EVALUATION OF TOTAL LYMPHOCYTE COUNT AND ABSOLUTE LYMPHOCYTE COUNT AS A SURROGATE MARKER FOR CD4 COUNT TO INITIATE ART

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CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF TOTAL LYMPHOCYTE COUNT AND ABSOLUTE LYMPHOCYTE COUNT AS A SURROGATE MARKER FOR CD4 COUNT TO INITIATE ART"** is the bonafide work of **Dr. VISHNU PRIYA S** in partial fulfilment of the university regulations of the Tamil Nadu Dr.M.G.R Medical University, Chennai, for M.D General Medicine Branch I examination to be held in April 2017.

Dr.V. T. Premkumar, M.D.
Professor and HOD,
Department of General Medicine,
Government Rajaji Hospital,
Madurai Medical College,
Madurai.

Dr.R.Prabhakaran, M.D.
Professor,
Department of General medicine
Government Rajaji Hospital,
Madurai Medical College,
Madurai.

Dean
Govt Rajaji Hospital,
Madurai.

DECLARATION

I,**Dr.VISHNU PRIYA S ,** solemnly declare that, this dissertation **"EVALUATION OF TOTAL LYMPHOCYTE COUNT AND ABSOLUTE LYMPHOCYTE COUNT AS A SURROGATE MARKER FOR CD4 COUNT TO INITIATE ART"** is a bonafide record of work done by me at the Department of General Medicine, Govt. Rajaji Hospital, Madurai, under the guidance of **Dr.R.PRABHAKARAN,M.D**. Professor,Department of General Medicine, Madurai Medical College, Madurai.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfilment of the rules and regulations for the award of **M.D Degree General Medicine Branch-I;** examination to be held in **April 2017.**

Place: Madurai

Date:

Dr.VISHNU PRIYA S

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INTRODUCTION

India has the third highest number of estimated people living with HIV in the world. National adult (15-49 years) HIV prevalence is estimated at 0.26%(0.22% to 0.32%) in 2015. The prevalence of HIV among males is 0.30% and among females is 0.22%. Among the states, Manipur remains the highest estimated HIV prevalence of 1.15% followed by Mizoram (0.8%), Nagaland (0.78%). Tamil nadu shows HIV prevalence greater than national prevalence (0.26%).

The cost of combination ART has dropped in recent years as a result of generic medicines and differential pricing based on country need and ability to pay. The cost of diagnostic services to determine eligibility for treatment and to monitor treatment response has kept ART inaccessible to many. The US Department of Health and Human services (DHHS) and the World Health Organisation (WHO) recommend initiating ART therapy based on consideration of a patient's CD4 T cell count when available.

The cost of monitoring HIV therapy may become more prohibitive than the cost of medications themselves. In December 2003, the WHO broadened the recommendations for initiation of ART when CD4 testing is unavailable to include WHO stage III or IV or WHO stage II in combination with a TLC 1200 cells/cu.mm. Many studies have evaluated the use of TLC as a surrogate marker for CD4 cell count with mixed results. Some studies have found a good relation but others have not. In

addition to low lymphocyte count ,anemia , thrombocytopenia have been associated with advanced HIV infection.

“The initiation of ART is based upon CD4 count <350 cells among patients in clinical staging1 and 2 according to NACO. The determination of CD4 count requires highly skilled laboratory personnel, sophisticated equipments which is difficult in resource limited localities. So the role of ALC and TLC in place of CD4 count is being studied and various cut offs have emerged. Many studies have suggested the use of ALC as a sensitive marker for HIV progression. Also,studies have demonstrated the usefulness of ALC or TLC in identifying patients who would benefit from initiating prophylaxis for AIDS related opportunistic infections”.

“The purpose of this dissertation is to assess the capability and clinical utility of TLC change to serve as a surrogate marker for CD 4 count change in monitoring patients, which has important implications for resource-limited settings. We are correlating CD4 Count to Total Lymphocyte Count, which is available in all resource limited settings as it is obtained by multiplying Total Leucocyte Count and percentage of Lymphocyte in Differential Count, to monitor disease progression in HIV infected persons”

“This dissertation is also an attempt to look into the correlation between hemoglobin and progression of the disease, which is monitored by change in CD 4 count . Since

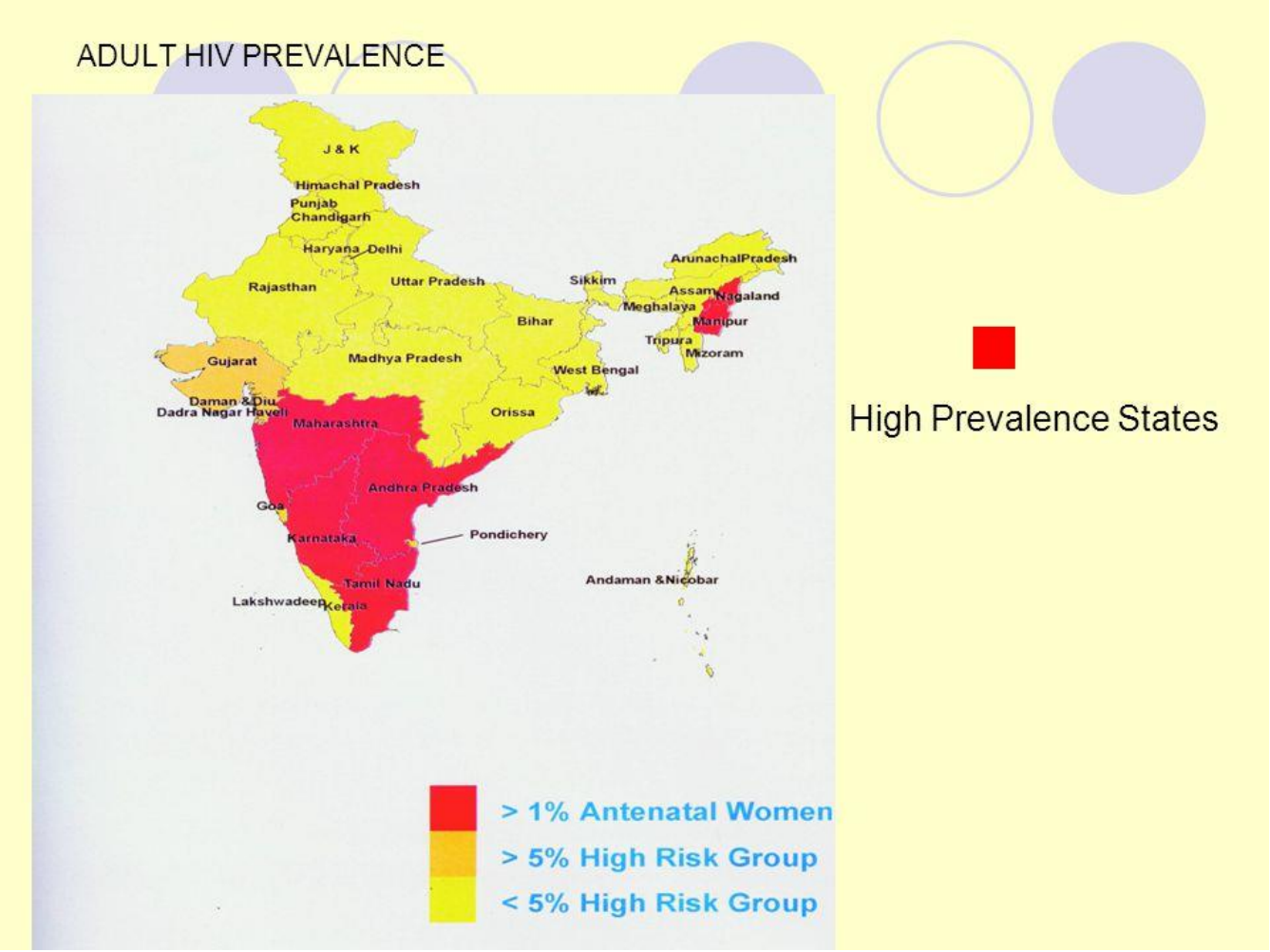
our study is being done in the resource poor set-up, the observations and conclusions can be used for monitoring progression of disease in HIV infected persons in our own set-up as well as other resource limited settings".

REVIEW OF LITERATURE

"AIDS is a retroviral disease caused by the Human immunodeficiency virus (HIV). It is characterised by infection and depletion of CD4 T lymphocytes , and by profound immune suppression leading to opportunistic infections , secondary neoplasm and neurological manifestations. Although AIDS was first described in the United States , it has now been reported in virtually every country in the world. Worldwide, more than 22 million people have died of AIDS since the epidemic was recognised in 1981 ; about 42 million people are living with the disease , and there are an estimated 5 million infections each year".

Global summary of the AIDS epidemic | 2015

Number of people living with HIV in 2015	Total	36.7 million [34.0 million – 39.8 million]
	Adults	34.9 million [32.4 million – 37.9 million]
	Women (15+)	17.8 million [16.4 million – 19.4 million]
	Children (<15 years)	1.8 million [1.5 million – 2.0 million]
People newly infected with HIV in 2015	Total	2.1 million [1.8 million – 2.4 million]
	Adults	1.9 million [1.7 million – 2.2 million]
	Children (<15 years)	150 000 [110 000 – 190 000]
AIDS deaths in 2015	Total	1.1 million [940 000 – 1.3 million]
	Adults	1.0 million [840 000 – 1.2 million]
	Children (<15 years)	110 000 [84 000 – 130 000]



EPIDEMOLOGY OF HIV IN INDIA

“India has a population of more than 1 billion people. Although only about 0.7% of its population is infected with HIV, it has more cases than any other country in the world, with more than 4.5 million HIV-seropositive patients. The epidemic of HIV/AIDS in India is distributed between the urban and rural populations mainly in the southern and western states of the country”

India has several different epidemics in various parts of the country. The epidemic in the western and southern states is primarily heterosexual. The north eastern states of India, being in geographical proximity to the Golden Triangle of Asia, initially experienced HIV in the injection drug user population and their sexual partners, but spread to the heterosexual population has been increasing. At present, the northern states, which are the most densely populated, appear to remain largely unaffected by the HIV epidemic.

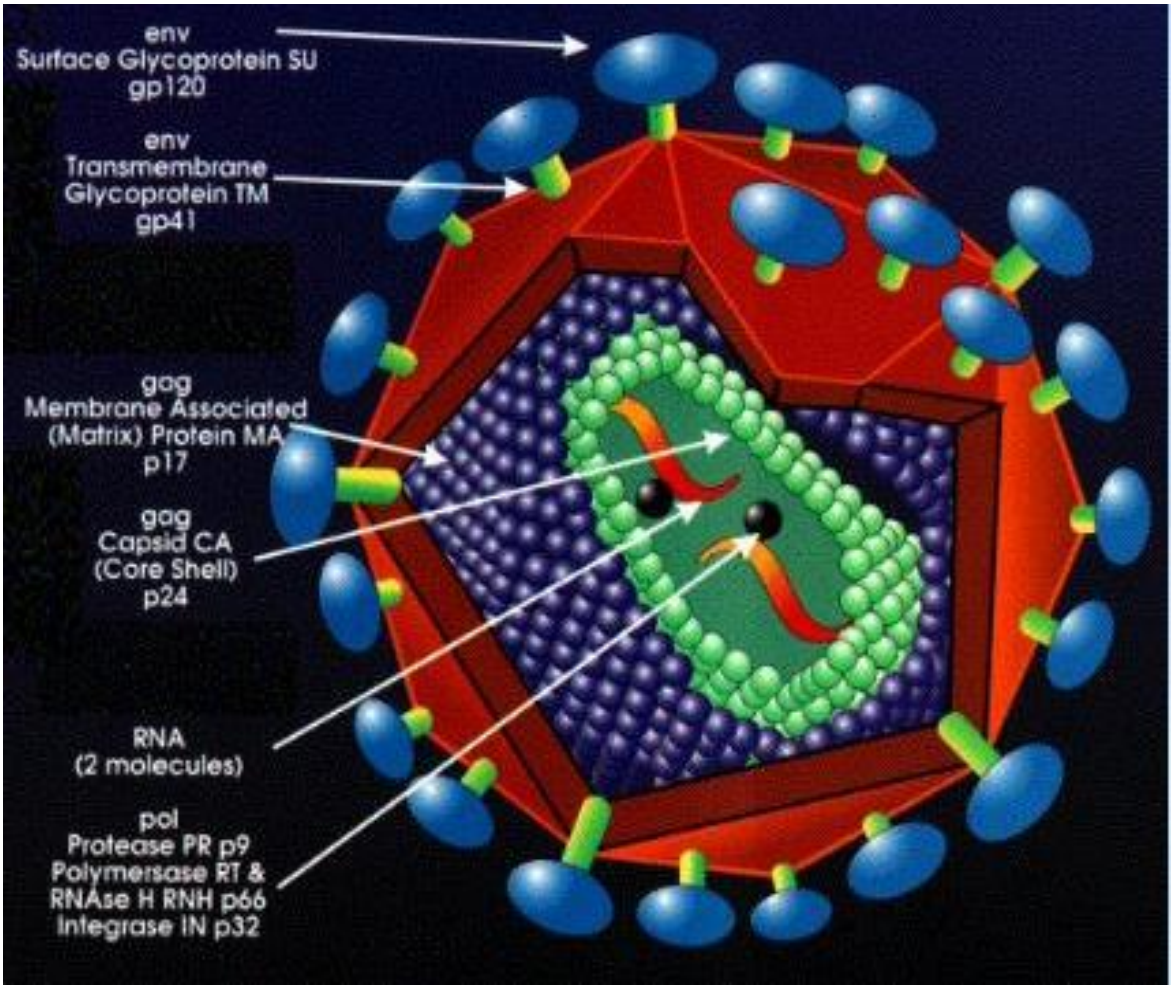
India has mounted a broad intervention program, including the government, and international, nongovernmental, and community-based organizations. The main barriers to effective control are insufficient resources, illiteracy, and stigma. Antiretroviral drugs are manufactured in the country and exported elsewhere, but their affordability (despite a drastic reduction in costs) and the feasibility of monitoring patients on drugs are in question.

HIV is the etiologic agent of AIDS; it belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses.The four retroviruses known to cause human disease belong to two distinct groups: the human T lymphotropic viruses (HTLV)-l and HTLV-2,which are transforming retroviruses; and the human immunodeficiency viruses,HIV- 1 and HIV-2, which cause cytopathic effects either directly or indirectly.The most common cause of HIV disease throughout the world and certainly in the United Statesis HIV- 1. The currently defined groups of HIV - 1 (M,N,O, P) and the HIV-2 groups A through H each are likely derived from a separate transfer to humans from a nonhuman primate reservoir. HIV -1 viruseslikely came from chimpanzees and/or gorillas, and HIV-2 from sooty mangabeys. The AIDS

pandemic is primarily caused by the HIV- 1 M group viruses. HIV-2 causes a similar disease principally in West Africa. Specific tests for HIV-2 are now available, and blood collected for transfusion is also routinely screened for HIV-2 seropositivity.

MORPHOLOGY OF HIV

" HIV virion is an icosahedral structure containing numerous external spikes formed by the two major envelope proteins,the external gp 120 and the transmembrane gp4 1 . The HIV envelope exists as a trimeric heterodimer. The virion buds from the surface of the infected cell and incorporates a variety of host proteins into its lipid. The virus core contains (1)major capsid protein p24, (2) nucleocapsid protein p7/p9, (3) two copies of genomic RNA , and (4) three viral enzymes (protease, reverse transcriptase , and integrase).p24 is the most readily detected viral antigen and is therefore the target for the antibodies used to diagnose HIV infection in blood screening . the viral core is surrounded by a matrix protein called p17 , lying beneath the virion envelope."



OUTSTANDING CHARACTERISTICS OF HIV:

“Predominantly affected cell: cells involved in immune function.

Permanent association of provirus within the infected cells.

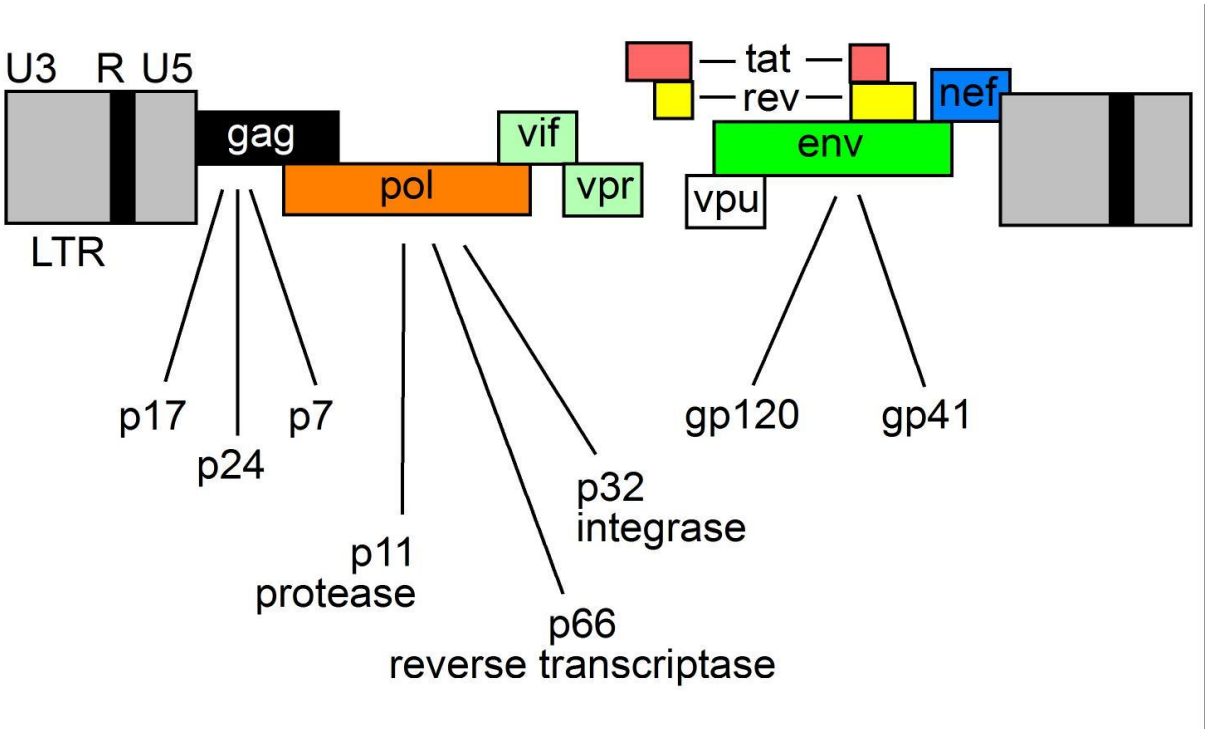
Expression of virus: restricted in some cells in vivo

Long clinical latency: disease progresses very slowly

Replication: species specific”

HIV GENOME:

“HIV genome comprises of 9 genes encoding 3 structural, 2 envelope, and 6 regulatory proteins. It also consists of a homodimer of linear, positive-sense, single-stranded RNA of approximately 9.2 kb in size. The key structural proteins are gag , pol ,env genes. The gag gene encodes the matrix. Nucleocapsid , capsid proteins. The pol gene encodes protease , reverse transcriptase and integrase. The env gene encodes a key”



“HIV surface antigen gp160 comprising of gp120 and gp41. The products of the gag and pol genes are translated initially into large precursor proteins that must be cleaved by the viral protease to yield the mature proteins.”

“Regulatory genes containinformation required for the production of proteins that control HIV’s ability to infect a cell, reproduce and cause disease. These are tat , rev, vif,nef,vpu,vpr. The **tatgene** –transactivator of transcriptionis encoded by 2 different exons from multiply spliced mRNA. The 102 aminoacidtat is responsible for activation of viral transcription through TAR binding, which creates binding sites for RNA pol II and other cellular proteins. It initiates synthesis of full-length transcripts. Tat is secreted into the circulation thus a possibility for inhibition by antibodies. It can also be a target for CTLs. Tat structure is rather conserved varying only slightly among different clades. It is produced in excess in infected cells.Tat also induces apoptosis of T cells, even the uninfected ones. It has also been shown to act as a neurotoxin and give rise to cells causing Kaposi’s sarcoma.”

“The rev geneis essential accessory protein whose function is to transport mRNA to the cytoplasm. Rev is a 117 aaphosphoprotein that binds to RRE (cis-acting element) within the env gene of all unspliced mRNAs. The N-terminal nuclear localization signal (NLS) directs its import back into the nucleus. Rev-dependent export of viral RNA distinguishes b/w early and late phase”.

“The **vif** gene is viral infectivity factor required for infection of human lymphocytes and some cell lines. Its ORF overlaps with the 3’ end of pol. A 23 kDa protein found

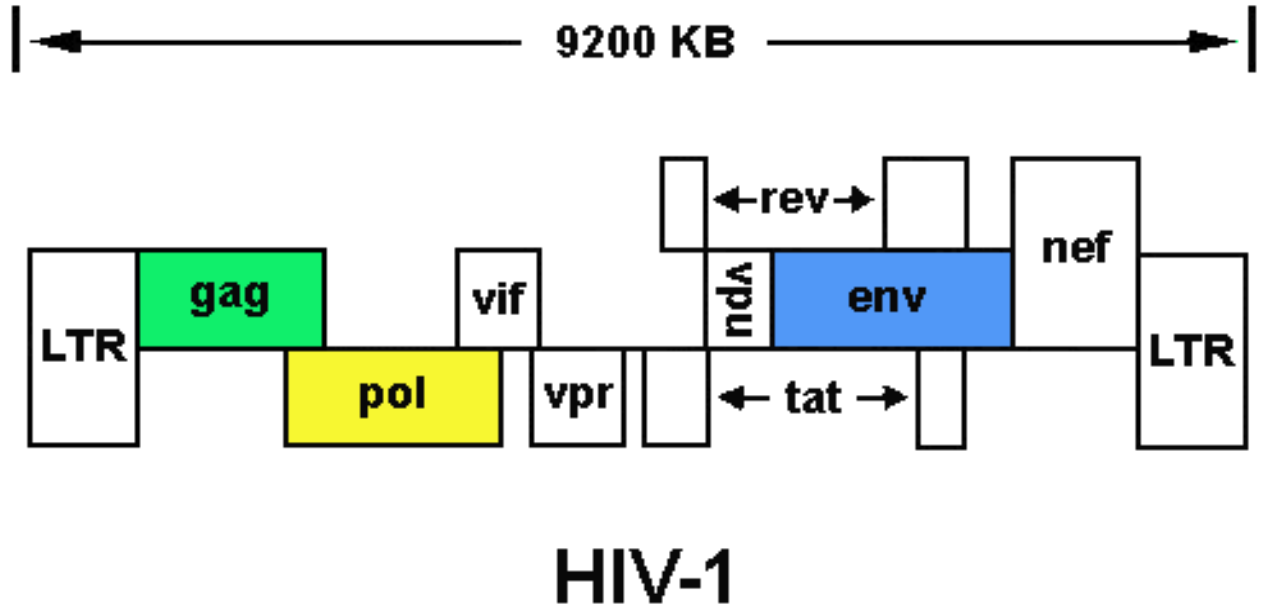
in the cytoplasm and cell membrane. The mechanism of action is not well understood but its importance in maturation process is recognized as the infectivity of Vif defective virions produced in non-permissive cells can be 25-100 times lower than wild type. Vif has been found in virus particles at levels similar to Pol but since it is also present in murine leukemia virus, possible significance of Vif incorporation is to be determined".

"The nef gene (negative factor) is a 27 kD determinant of progression to AIDS. It downregulates cell surface receptor expression, interferes with signal transduction pathways and enhances viral infectivity and production. Nef is post-translationally modified by phosphorylation and by the irreversible attachment of myristic acid to its N-terminus, which targets Nef to the cellular membrane. The most enigmatic HIV protein as its mechanisms of action are not well understood and many contradictory phenotypes have been associated with expression of Nef."

"The vpu gene (viral protein U), is 81 aa membrane protein expressed as part of a bicistronic message also encoding Env and regulated by Rev. It promotes release of viral particles from plasma membrane of infected cell and degrades CD4in the endoplasmic reticulum. The ability to form a cation-selective ion channel has also been described as another function of Vpu but its role is not known."

"The vpr gene (viral protein R), is 96 aa, 14 kDa protein responsible for G2 cell cycle arrest thought to indirectly enhance viral replication by increasing transcription from LTR. Vpr expression causes breaks in the nuclear lamin structure, which weakens nuclear envelope and interferes with DNA synthesis thus cycle arrest prior to mitosis.

It is also implicated in facilitating infection of non-dividing cells, mostly macrophages. Vpr also functions to connect the pre-integration complex to the cellular nuclear import machinery.”



HIV-1

PROTEINS WITH ANTIVIRAL ACTIVITIES IN HUMAN CELLS

1. APOBEC3G

2. TRIM5α

3. TETHERIN

“APOBEC3G

It causes suppression of viral transcription. It acts on viral genome and substitute adenosine in the place of guanine in the viral genome. The virus can easily overcome its action by ubiquitinisation and degradation of the protein. This is carried out by vif gene”.

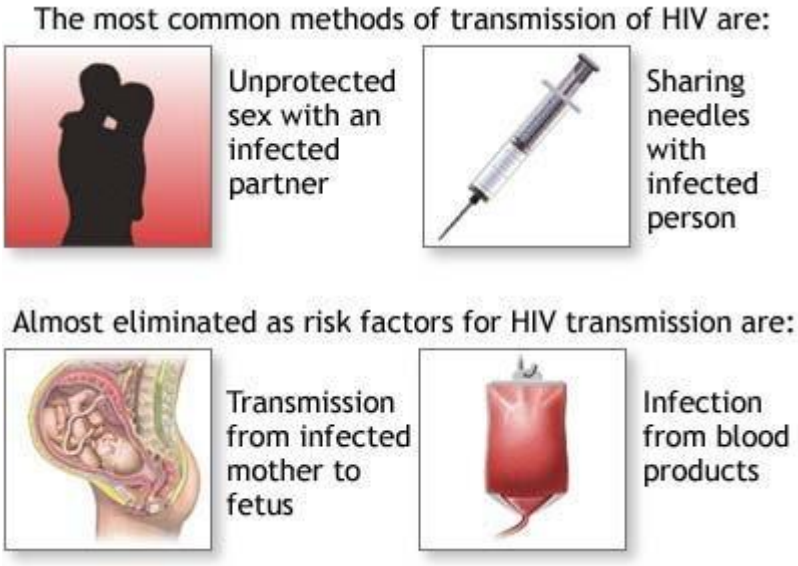
TRIM5α

“This protein causes premature uncoating of viral nucleocapsid iin cellular cytoplasm itself. Virus evades this cellular antiviral mechanism by producing variation in capsid protein.”

TETHERIN

“This is a newly found molecule present in the host cytoplasm. It is otherwise called CD317. It inhibits budding of newly formed virus through cellular membrane. Viruses are sequestered in tetherin mediated vesicles. Action of tetherin is inhibited by increased production of vpu protein by the virus.”

MODES OF TRANSMISSION



“HIV is transmitted primarily by sexual transmission both by heterosexual and male to male transmission,by blood products,and by infected mother to child or via breast milk.”

SEXUAL TRANSMISSION

“The most frequent mode of transmission of HIV is through sexual contact with an infected person. The majority of all transmissions worldwide occur through heterosexual.however, the pattern of transmission varies significantly among countries. In the United States, as of 2010, most common mode of transmission is male to male transmission. In India most common mode of transmission is through

heterosexual.Women of childbearing age are at particular risk for acquiring HIV through unprotected sex”

“Risk of transmission increases in the presence of many sexually transmitted infections and genital ulcers .The viral load of infected person is an important risk factor in both sexual and mother-to-child transmission.The rate of HIV transmission is highest (twelve fold)during early stages of HIV infection. If the person is in the late stages of infection, rates of transmission are approximately eightfold greater.”

TRANSMISSION BY BLOOD AND BLOOD PRODUCTS

“HIV can be transmitted to individuals who receive HIV tainted blood transfusions, blood products, or transplanted tissue as well as to IDUs who are exposed to HIV while sharing injections . It is estimated that >90% of individuals exposed to HIV contaminated blood products become infected. In some resource poor countries due to inadequate screening HIV continues to be transmitted by blood , blood products and tissues .”

OCCUPATIONAL TRANSMISSION OF HIV

“Health care workers and laboratory personnel who are working with HIV containing materials are at risk. Exposures that place a health care worker at potential risk of HIV

infection are percutaneous injuries or contact of mucous membrane or nonintact skin with blood ,tissue or other infectious body fluids.”

TRANSMISSION BY ORGAN TRANSPLANTATION

“Hiv transmission has been reported in all types of solid organ transplantion. Oragan donation by hiv patients has been prohibited world wide. There is growing debate to life this ban thereby allowing hiv”

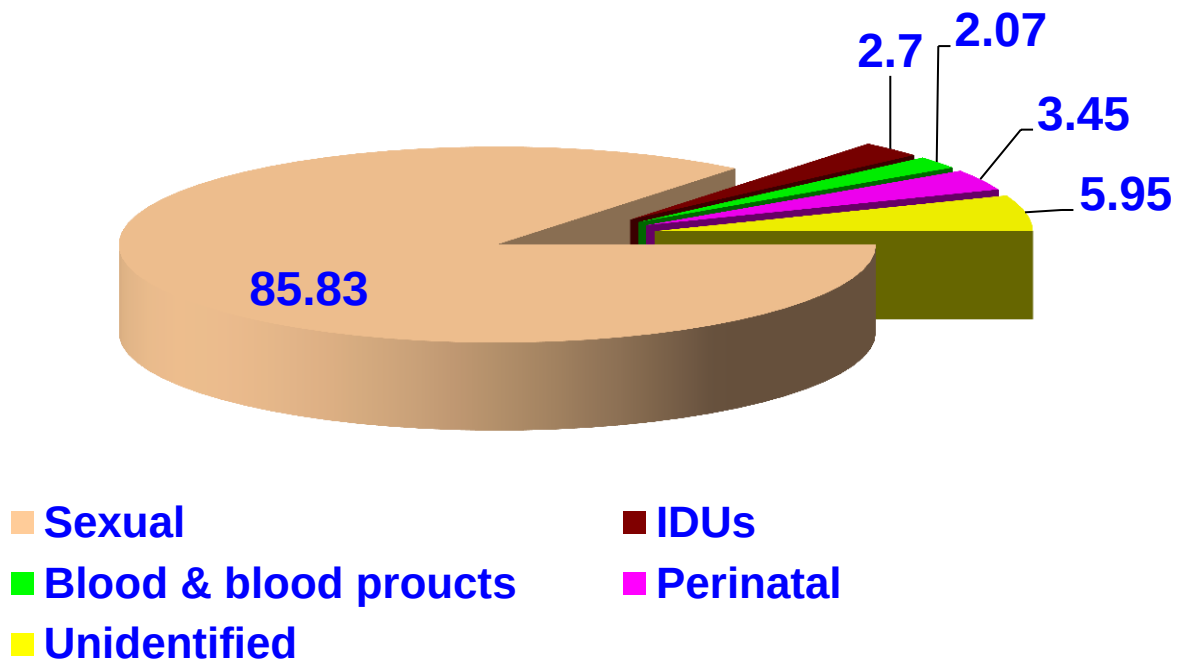
MATERNAL –FETAL/INFANT TRANSMISSION

“HIV infection can be transmitted from an infected mother to her fetus during pregnancy,during delivery or by breast feeding. However , maternal transmission to the fetus occurs most commonly in the perinatal period. The risk factors for mother to child transmission of HIV via breast feeding are low maternal CD4 T cell count , maternal vitamin A deficiency, presence of HIV in breast milk, and presence of mastitis. The risk is highest during the early months of breast feeding.”

TRANSMISSION BY OTHER BODY FLUIDS

“The following fluids are considered infectious : cerebrospinal fluid , synovial fluid , pleural fluid, peritoneal fluid , pericardial fluid and amniotic fluid.”

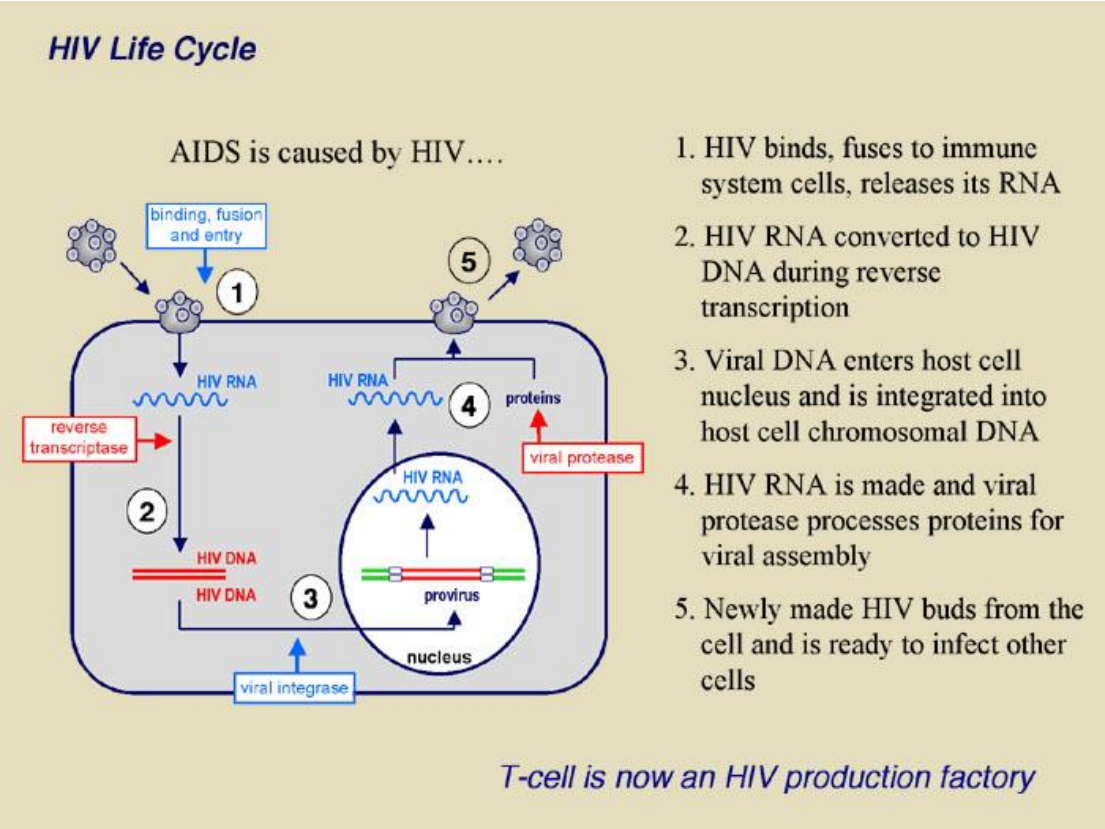
MODE OF HIV TRANSMISSION IN INDIA :



LIFE CYCLE OF HIV:

“The entry of HIV into the cells requires the CD4 molecule , which acts as a high affinity receptor for the virus. This explains the tropism of the virus for CD4 +T cells ,particularly macrophages and DCs. However , binding to CD4 is not sufficient for

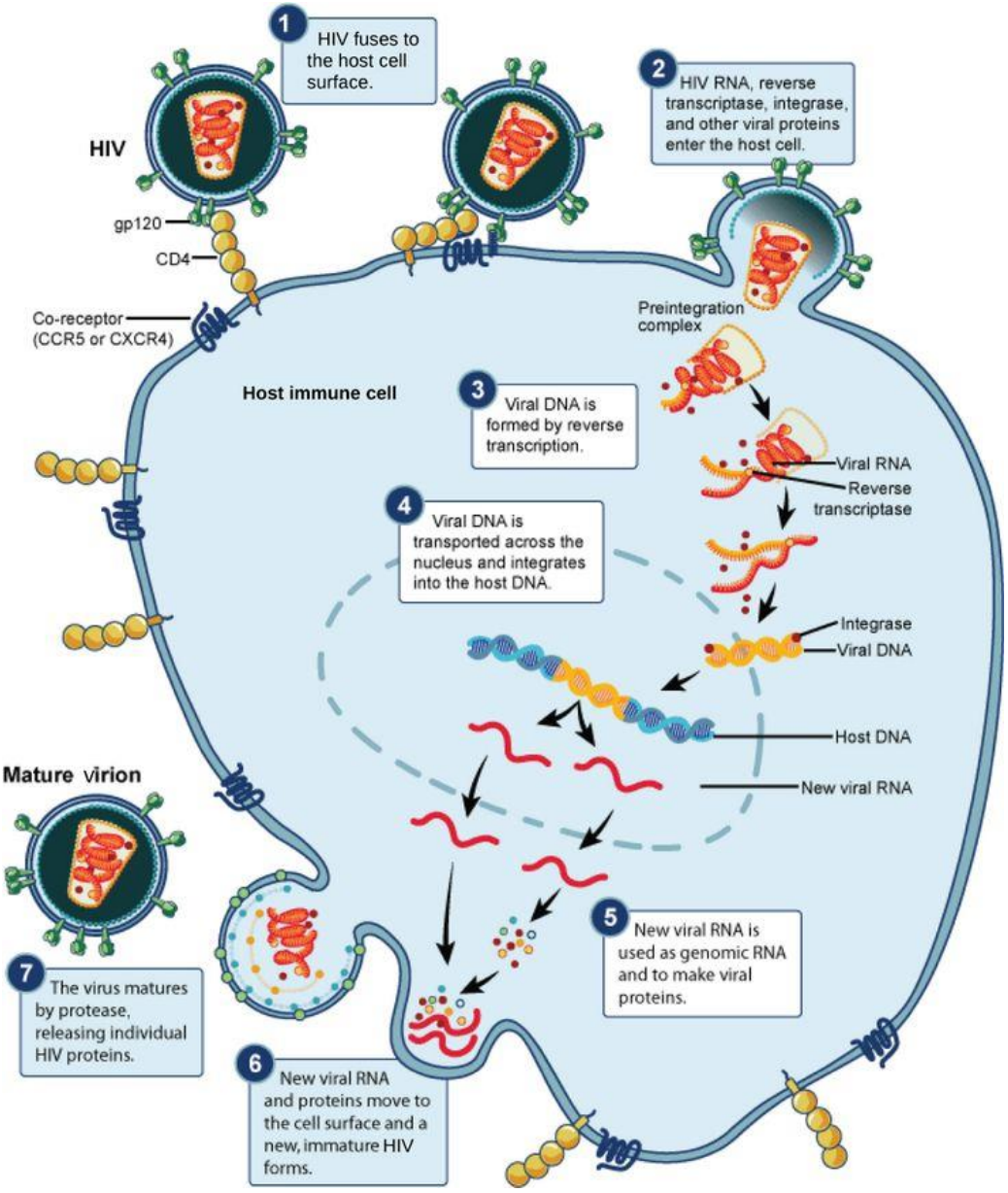
infection; the HIV envelope gp 120 must also bind to other cell surface molecules (coreceptors) to facilitate cell entry. Two cell surface chemokine receptors, CCR5 and CXCR4, serve this role."



"HIV envelope gp120 binds initially to CD4 molecule. This binding leads to a conformational change that exposes a new recognition site on gp120 for the CXCR4 or CCR5 coreceptors. The gp41 then undergoes a conformational change that allows it

to insert into the target membrane , and this facilitates fusion of the virus with the cell.”

“The coreceptors are critical components of the HIV infection process. HIV strains could be classified according to their relative ability to infect macrophages and/or CD4 T cells. Macrophage tropic (R5 virus) strains infect both monocytes/macrophages and freshly isolated peripheral blood T cells,whereas T cell tropic (X4 virus) strains infect only activated T cell lines.”

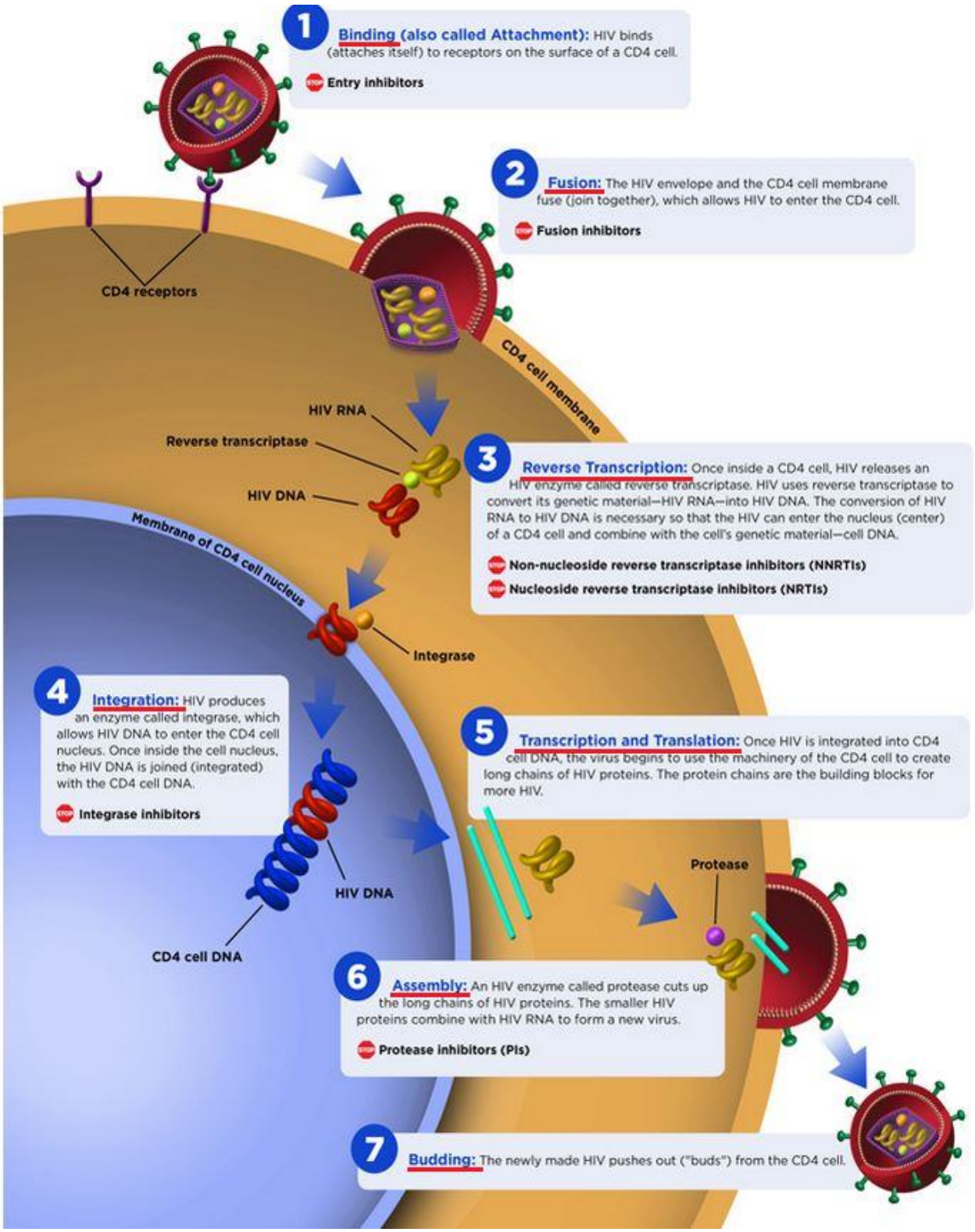


“R5 strains use CCR5 as their coreceptor ,and.because CCR5 is expressed on both monocytes and T cells, these cells succumb to infection by R5 strains. Conversely , X4 strains bind to CXCR4 ,which is expressed on T cell lines , so that only activated T cells are susceptible”.

“Approximately 90% of HIV infections are initially transmitted by R5 strains. Over the course of infection, X4 viruses gradually accumulate . These are virulent and are responsible for T cell depletion in the final rapid phase of disease progression.”

“During the course , R5 strains evolve into X4 strains , as a result of mutations in genes that encode gp120. The resultant transition in the ability of the virus to bind CXCR4 but not CCR5 is important in pathogenesis of AIDS because T tropic viruses are capable of infecting naïve T cells and thymic T cell precursor and cause greater T cell depletion and impairment.”

“Once internalized , the viral genome undergoes reverse transcription leading to the formation of complementary DNA. In quiescent T cells , HIV proviralcDNA may remain in the cytoplasm in a linear episomal form. The cDNA enters the nucleus in dividing T cells and becomes integrated into the host genome. After integration ,the provirus may remain non transcribed for months or years , and the infection becomes latent. Alternatively ,proviral DNA may be transcribed to form complete viral particles that bud from the cell membrane.”


1
Binding (also called Attachment): HIV binds (attaches itself) to receptors on the surface of a CD4 cell.
Entry inhibitors
2
Fusion: The HIV envelope and the CD4 cell membrane fuse (join together), which allows HIV to enter the CD4 cell.
Fusion inhibitors
CD4 receptors
CD4 cell membrane
HIV RNA
Reverse transcriptase
HIV DNA
3
Reverse Transcription: Once inside a CD4 cell, HIV releases an HIV enzyme called reverse transcriptase. HIV uses reverse transcriptase to convert its genetic material—HIV RNA—into HIV DNA. The conversion of HIV RNA to HIV DNA is necessary so that the HIV can enter the nucleus (center) of a CD4 cell and combine with the cell's genetic material—cell DNA.
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
Nucleoside reverse transcriptase inhibitors (NRTIs)
Membrane of CD4 cell nucleus
Integrase
4
Integration: HIV produces an enzyme called integrase, which allows HIV DNA to enter the CD4 cell nucleus. Once inside the cell nucleus, the HIV DNA is joined (integrated) with the CD4 cell DNA.
Integrase inhibitors
5
Transcription and Translation: Once HIV is integrated into CD4 cell DNA, the virus begins to use the machinery of the CD4 cell to create long chains of HIV proteins. The protein chains are the building blocks for more HIV.
HIV DNA
CD4 cell DNA
Protease
6
Assembly: An HIV enzyme called protease cuts up the long chains of HIV proteins. The smaller HIV proteins combine with HIV RNA to form a new virus.
Protease inhibitors (PIs)
7
Budding: The newly made HIV pushes out ("buds") from the CD4 cell.

“Untreated HIV infection results in the progressive loss ofCD4+ cells from the circulation as well asdepletion of CD4+ cells from total body. There are several plausibletheories to explain the CD4+ cell depletionseen in HIV disease. CD4+ cells may be depleted because they are destroyed (the high-turnover model) or because their production is impaired (the regenerative failure model) .Another theory , holds that thefraction of circulating cells may decrease-- giving the appearance of loss—if HIV infection results in their redistribution out ofthe peripheral blood and into the confinesof lymphoid organs”.

“A critical distinction between the models for high turnover and regenerative failure is that the latter includes a primary pathogenic event in HIV disease that rests upon a failure to produce new CD4+ (and CD8+) cells . The modelspecifies that the sources of production—namely, the bone marrow, the thymus, and other extrathymic lymphoid organs(e.g., lymph nodes, spleen, and mucosa)—are rendered dysfunctional in later stages of HIV disease, resulting in low (or no) rates of replacement in the face of continued CD4+ cell destruction. Over time, this imbalance leads to a decrease in the totalbody pool of mature T-cells and to immune system collapse, whether or not the destruction rate of CD4+ cells is accelerated. With the initiation of HAART, the viral assault on progenitor cells is halted, allowing for the production of new T-cells. Coupled with diminished destruction of mature T-cells, the CD4+ cell count rises.”

“When HIV is introduced into the body, it is probably concentrated with draining lymph nodes and presented as an antigen, resulting in enhanced movement of T-cellsinto nodes and vigorous T-cell proliferation. The high turnover model holds that immune activation caused by HIV will be associated, by direct and indirect means, with accelerated destruction of T-cells and that the initiation of highly active antiretroviral therapyslows T-cell turnover and with it a decrease in the death rate of CD4+ cells. The regenerative failure model specifies that the source of T-cell productionnamely the bone marrow, the thymus, and other extrathymic lymphoid organs are rendered dysfunctional in later stages of HIV disease, resulting in low (or no) rates of replacement in the face of continued CD4+ cell destruction. Over time, this imbalance leads to a decrease in the total pool of mature T-cellsand to immune system collapse, whether or not the destruction rate of CD4+ cells is accelerated. With the initiation of HAART, the viral assault on progenitor cells is halted, allowing for the production of new T-cells. Coupled with diminished destruction of matureT-cells, the cd4+ cell count rises”.

ACCLERATED DESTRUCTION AND REGENERATIVE FAILURE MODELS OF HIV

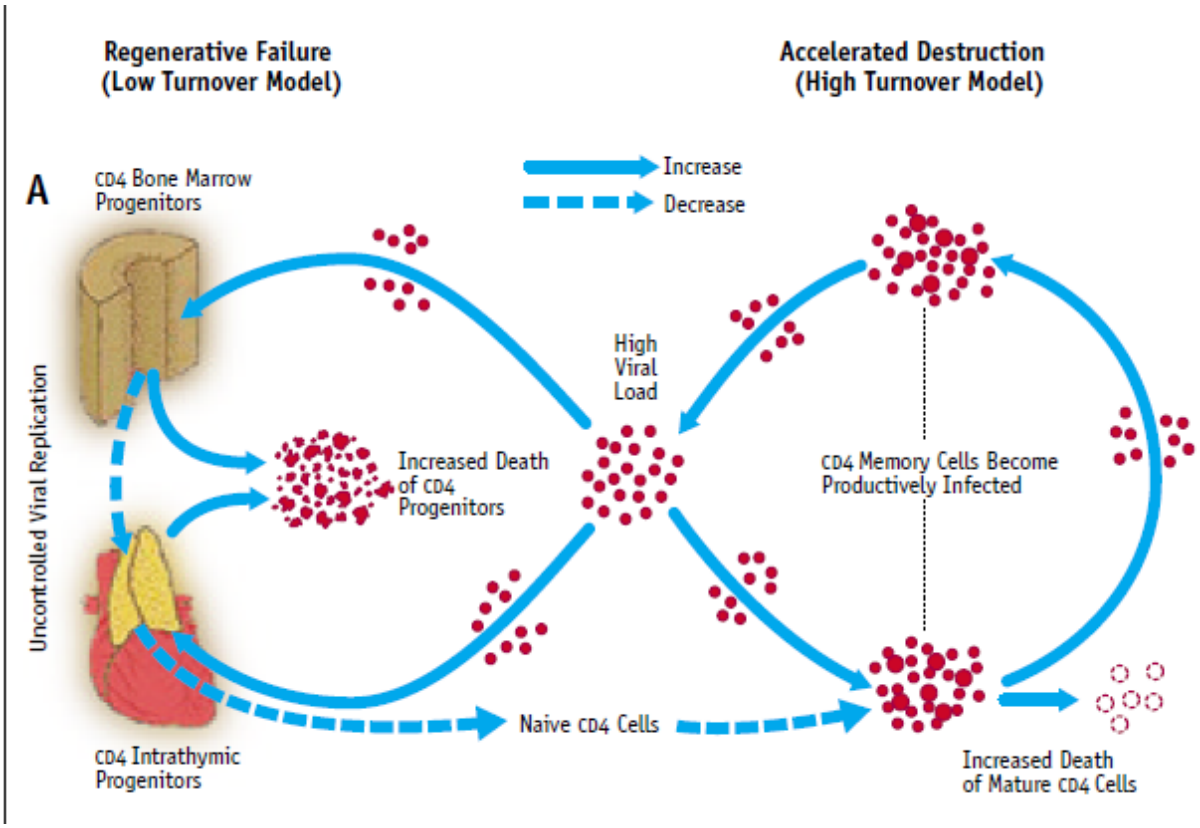


TABLE 1. Mechanisms of CD4+ Cell Depletion: Destruction of Mature CD4+ Cells

Direct destruction of infected cells

- Envelope-mediated apoptosis
- Vpr-induced G2 arrest and apoptosis
- Disruption of cell membrane integrity/syncytia formation
- Accumulation of unintegrated viral DNA

Indirect induction of death in uninfected cells

- Cytolysis by HIV-specific cytolytic T-cells or by natural killer cells
- Autoimmune reactions of a humoral or cellular nature
- Incorporation into syncytia by neighboring infected cells
- Triggering of apoptosis upon cell activation or cross-linking of CD4-bound gp120
- Enhanced HIV transmission and/or apoptosis following interaction with nearby infected antigen-presenting cell

TABLE 2. Mechanisms of CD4+ Cell Depletion: Impaired T-Cell Production

Direct effects of virus

- Infection-mediated death of progenitor cells
- Destruction of the supporting stromal network required for multilineage or lineage-restricted hematopoiesis

Indirect effects of virus

- Cytokine dysfunction
- Opportunistic infections of bone marrow (e.g., CMV or MAC)
- HIV-induced apoptosis
- Infiltrating malignancies
- Myelotoxic effects of drugs
- Deficiences of vitamins and of other essential factors

DIAGNIOSIS OF HIV FUNCTION

“HIV infection is identified either by the detection of HIV-specific antibodies in serum or plasma or by demonstrating the presence of the virus by nucleic acid detection using polymerase chain reaction (PCR), p24 antigen testing or, rarely these days, by growing virus in cell culture. Antibody testing is the method most commonly used to diagnose HIV infection. With the highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) tests currently on the market, seroconversion can be detected within two to three weeks of infection in the majority of cases. In a small number of early seroconverters who are still in the 'window period', the p24 antigen may become positive before antibody is detectable. Therefore, to enable the laboratory to select appropriate testing, it is important to provide a clinical history that includes any recent high-risk behaviour or symptoms consistent with sero conversion illnes.”

Diagnostic Tests for HIV Infection

- Serological methods for detection of antibody
  - Rapid tests
  - ELISA
  - Western blot
- Antigen detection methods
  - P24 antigen capture test
  - Polymerase Chain Reaction (also known as PCR or viral load)

4

"All HIV diagnostic laboratories should confirm repeatedly positive EIA screen tests with another assay. The Western blot - the most commonly used confirmatory test - is a highly specific immunoblot that allows for the visualization of antibodies to the structural polypeptides of HIV. Some laboratories may use a radioimmuno precipitation assay as their confirmatory assay or as part of their HIV testing algorithm. In a radioimmunoprecipitation assay test, radiolabelled viral proteins are reacted with the patient's serum to produce radioactive antigen-antibody complexes."

ANTIBODY DETECTION BY EIA

“EIA is commonly used as a screening assay for HIV. These assays are used because they are highly sensitive and generally amenable to automation, facilitating high-volume testing. HIV This has shortened the 'window period', or the time from exposure to seroconversion, from up to 12 weeks or more in the early days of diagnostic testing to the current 'window period' of less than three weeks in most cases.”

“The small disadvantage of such a highly sensitive test is that the test produces false positives, the number and type of which vary with the assay used and the HIV prevalence in the tested population. All HIV diagnostic laboratories must confirm repeated EIA screen-positive results by a confirmatory assay, usually with Western blot.”

P24 ANTIGEN

“p24 antigen tests are also EIA-based and use antibody to capture the disrupted p24 antigen from patient serum. Positive results that are repeatable must be confirmed with a neutralization procedure. In rare instances, the p24 antigen can be detected before HIV antibody in newly infected individuals. This test is useful for specimens from patients that are high risk and symptomatic but HIV EIA-negative, or for specimens that are EIA-positive but Western blot-negative or -indeterminate . A follow-up HIV antibody test should be requested when a patient is p24 antigen-positive but antibody-negative. In a seroconverting patient, the follow-up specimen

will be positive within a few weeks after the initial screen. It is important to remember that not all seroconverting patients will have detectable p24 antigen, and that this antigen may not be reliably found in individuals who are known to be HIV antibody-positive."

WESTERN BLOT

"The most commonly used confirmatory test is western blot. This assay takes advantage of the fact that multiple HIV antigens of different , well characterised molecular weights elicit the production of specific antibodies. These antigens can be separated on the basis of molecular weight, and antibodies to each component can be detected as discrete bands on the western blot."

LABORATORY MONITORING OF PATIENTS WITH HIV INFECTION

"The close relationship between clinical manifestations of HIV infection and CD4 T cell has made measurement of the latter a routine part of the evaluation of HIV infected individuals. Determination of CD4 T cell count and measurements of the levels of HIV RNA in serum or plasma provide a powerful set of tools for determining prognosis and monitoring response to therapy."

CD4 CELL COUNT

“CD 4 count analysis is the best indicator of the immediate state of immunological competence of the patient with HIV infection. This measurement , which can be made directly or calculated as the product of the percent of CD4 T cells (determined by flow cytometry) and the total lymphocyte count (determined by the white blood cell count (WBC) and the differential percent) has been shown to correlate well with the level of immunological competence.”

“Patients with CD4 T cell count <200/µL are at high risk of disease from P.jiroveci , while patients with CD4 T cell count <50/µL are at high risk of disease from CMV , mycobacteria of the M.avium complex and/or T.gondii.”

“Patients with HIV infection should have CD4 T cell measurement performed at the time of diagnosis and every 3-6 months thereafter. According to the guidelines, a CD4 T cell count <350/µL is an indication for initiating ART treatment and decline in CD4 T cell count of 25% is an indication for considering change in therapy.”

“Patients with CD4 count <200/µL should be placed on prophylaxis regimen for P.jiroveci and once the count is <50/µL primary prophylaxis for MAC infection is indicated. In patients with hypersplenism or who have undergone splenectomy the CD4 cell percentage may be reliable than CD4 cell count. A CD4 cell percent of 15 is comparable to a CD4 T cell count of 200/µL.”

CD4 LEVELS IN RELATION TO SEVERITY OF IMMUNOSUPPRESSION

Not significant immunosuppression	>500 cells/μL
Mild immunosuppression	350-499 cells/μL
Advanced immunosuppression	200-349 cells/μL
Severe immunosuppression	<200 cells /μL

HIV RNA DETERMINATIONS

“HIV RNA measurements has become important component in the monitoring of patients with HIV infection. The two most commonly used techniques are the RT-PCR assay and the bDNA assay. Quantitative RNA PCR must only be used to monitor HIV-positive individuals before or during antiretroviral therapy. It is used in conjunction with CD4 counts and general clinical assessments to ascertain when therapy should be started. It is also used to help determine the patient's response to therapy. Therapy should be considered in patients with >100,000 copies of HIV RNA per millilitre”.

“HIV RNA measurements are to be done frequently to monitor therapy , which has great economic burden to many people in developing countries. Therefore other simple clinical or laboratory parameter to monitor therapy is essential in developing countries.”

OTHER TESTS

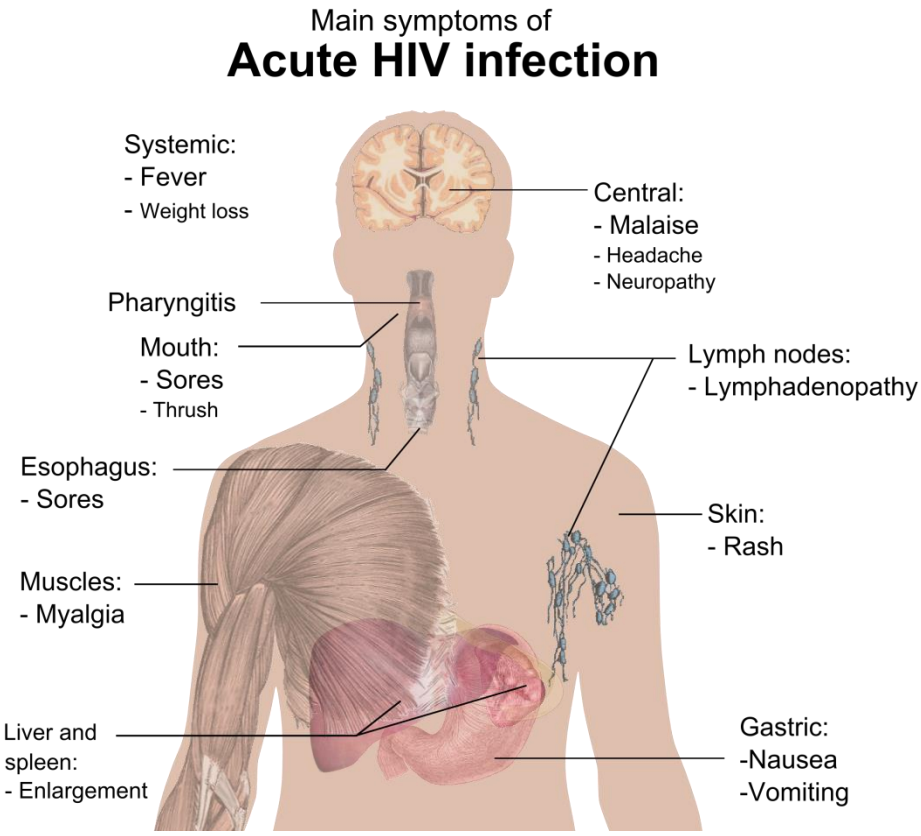
“A variety of other laboratory tests have been studied as potential markers of HIV disease activity. Among these are quantitative culture of replication – component HIV from plasma , peripheral blood mononuclear cells , or resting CD4 T cells , circulating levels of β2microglobulin , soluble IL-2 receptor, Ig A , acid labile endogenous interferon orTNF a and the presence or absence of activation markers such as CD38, HLA-DR. Though these are the markers of disease activity, they do not play a major role in monitoring of patients with HIV infection.”

“Hence in our study various clinical and inexpensive laboratory measures such as clinical staging WHO , total lymphocyte count (TLC), haemoglobin , absolute lymphocyte count (ALC) were done to assess HIV disease activity and compared with CD4 count. Then , it was analysed that these parameters may be used as a surrogate marker for CD4 cell count to initiate ART and to monitor therapy.”

CLINICAL MANIFESTATIONS

“The clinical consequences of HIV infection encompass a spectrum ranging from an acute syndrome associated with primary infection to a prolonged asymptomatic state to advanced disease. It is best to regard HIV disease as beginning at the time of primary infection and progressing through various stages.”

ACUTE HIV SYNDROME



PERSISTENT GENERALISED LYMPHADENOPATHY

“It is unknown how many individuals infected with HIV will have signs and symptoms of an acute viral infection.This illness is similar to Infectious mononucleosis and presents with fever, myalgia, nausea, and pharyngitis 1to 8 week after exposure. On examination, macular rash, athralagia, oral ulceration, lymphadenopathy and occasional hepatosplenomegaly can be noted. Some cases present with symptoms of aseptic meningitis, fever, headache, meningismus and photophobia. CSF pleocytosis can be present and HIV has been detected in CSF. The acute illness resolves spontaneously after 1 to 2 weeks and is diagnosed by the presence of an HIV core antigen called p24 in the serum.Following this acute illness, patients can remain in a latent asymptomatic state for years.”

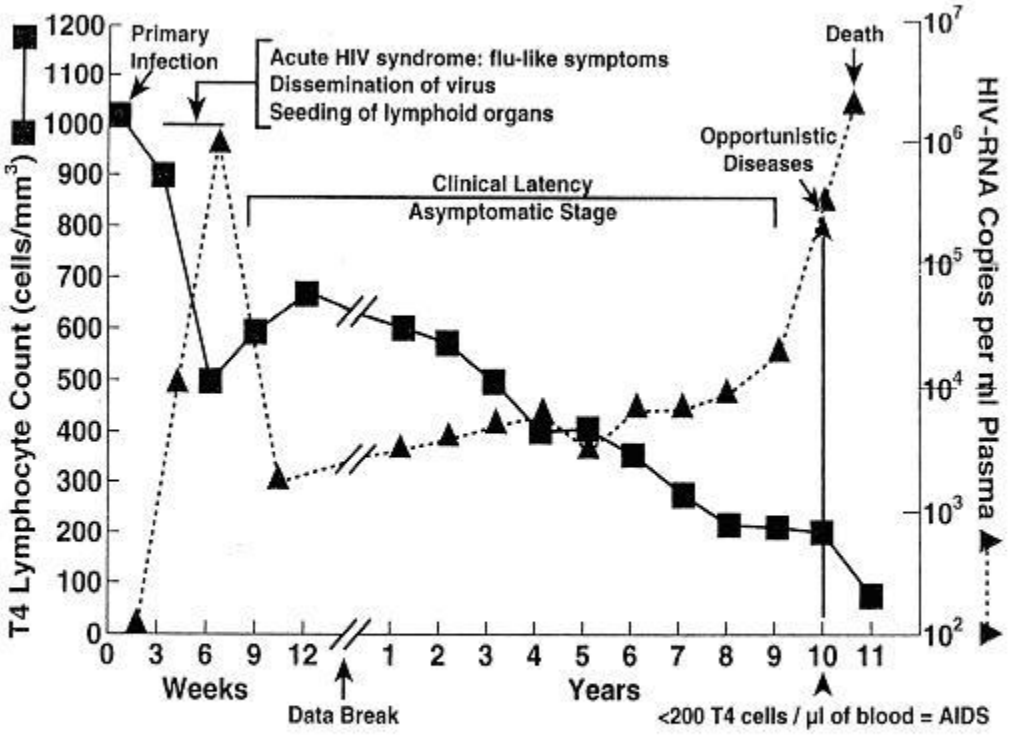
“Some HIV-infected individuals develop persistent generalized lymphadenopathy. This entity is a frequent early clinical sign of HIV infection and appears to follow seroconversion in 50% of individuals.”

ASYMPTOMATIC STAGE – CLINICAL LATENCY

“The length of time from initial infection to the development of clinical disease varies for a period of 10 years.HIV disease with active viral replication is ongoing and progressive during this period. The middle chronic phase represents a stage of relative containment of the virus. The immune system is intact at this point but there is continued HIV replication that may last for several years. Patients either are asymptomatic or develop persistent lymphadenopathy and many patients have minor opportunistic infections. During this phase , viral replication in the lymphoid tissue

continues unabated. The extensive viral turn over is associated with continued loss of CD4 Cells , but there is replenishment of CD4 cells .So the loss is modest."

The typical clinical course of HIV disease.



SYMPTOMATIC DISEASE

"Symptoms of HIV disease can appear at any time during the course of HIV infection. The spectrum of illnesses changes as the CD4+ T cell count declines. The more severe and life-threatening complications of HIV infection occur in patients with CD4+ T cell counts <200/µL. A diagnosis of AIDS is made in individuals age 6 years and older with HIV infection and a CD4+ T cell count <200/µL and in anyone with HIV

infection who develops one of the HIV -associated diseases considered to be indicative of a severe defect in cell mediated immunity. The causative agents of the secondary infections are opportunistic organisms such as P. jiroveci, atypical mycobacteria,CMV, and other organisms that do not ordinarily cause disease in the absence of a compromised immune system."

TABLE 1. REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR ADULTS AND ADOLESCENTS

Primary HIV infection
Asymptomatic
Acute retroviral syndrome
Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy (PGL)
Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulcerations
Papular pruritic eruptions
Seborrhoeic dermatitis
Fungal nail infections of fingers

Clinical stage 3

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

- Severe weight loss (>10% of presumed or measured body weight)
- Unexplained chronic diarrhoea for longer than one month
- Unexplained persistent fever (intermittent or constant for longer than one month)
- Oral candidiasis
- Oral hairy leukoplakia
- Pulmonary tuberculosis (TB) diagnosed in last two years
- Severe presumed bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary

- Unexplained anaemia (<8 g/dl), and or neutropenia (<500/mm^3) and or thrombocytopenia (<50 000/ mm^3) for more than one month

Clinical stage 4

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)
- Oesophageal candidiasis
- Extrapulmonary TB
- Kaposi's sarcoma
- Central nervous system (CNS) toxoplasmosis
- HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary:

Extrapulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacteria infection
Progressive multifocal leukoencephalopathy (PML)
Candida of trachea, bronchi or lungs
Cryptosporidiosis
Isosporiasis
Visceral herpes simplex infection
Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
Recurrent non-typhoidal salmonella septicaemia
Lymphoma (cerebral or B cell non-Hodgkin)
Invasive cervical carcinoma
Visceral leishmaniasis

"The proposed revisions of WHO staging are designed to

to guide decisions on when to start cotrimoxazole prophylaxis, start ART and other HIV related interventions"

"provide simple guidance to assist clinical care providers on when to start, substitute, switch or stop ART in HIV-infected adults and adolescents, or to trigger referral as outlined in the WHO ART guidelines for a public health approach

be used to assess current clinical status of individuals in HIV care, either on or off ART"

"encourage clinical care providers to offer diagnostic testing for HIV in adults and adolescents exhibiting the clinical events suggestive of HIV disease prompt urgent offer of HIV diagnostic testing for stage 3 or stage 4 events either on site, or by referral for testing to a site where immediate assessment by HIV care providers able to initiate ART can be performed"

"be used to guide clinicians in assessing the response to ART, particularly where viral load and/or CD4 counts or percentages are not widely or easily available (new or recurrent stage 4 events may suggest failure of response to treatment; new or recurrent stage 2 or stage 3 events may suggest an inadequate response to treatment, potentially because of poor adherence; however further evidence is required in order to determine the significance of staging events once ART has commenced. Clinical events in the first three months after ART has begun may be caused by immune restoration syndrome (IRS) rather than a poor response to ART)."

HIV AND METABOLIC SYNDROME:

“Prevalence of metabolic syndrome amonh hiv infected patients around 20%.Hiv causes dyslipidemia,increases triglycerides and very low density lipoprotein.Protease inhibitors also causes insulin resistance leading to metabolic syndrome.Hiv leads to lipodystrophy promoting visceral adiposity causing insulin resistance
Due to increased prevalence of metabolic syndrome in hiv they are more prone for cardiovascular disease,early atherosclerosis,diabetes related complications”

HIV AND DIABETES:

“HIV patients present with metabolic syndrome, altered glucose metabolism, dyslipidemia and lipodystrophy”.

“RISK FACTORS: These include advancing age, male gender, longer duration of HIV infection, high CD4 count, high viral burden, high body mass index, greater waist circumference or waist- to- hip ratio.”

Impaired glucose tolerance, and insulin resistance are noted to precede weight loss in patients with HIV . Insulin resistance, rather than insulin deficiency, is usually implicated in the pathogenesis of diabetes in HIV-infected patients.

“Autoimmune diabetes, however, has recently been reported to develop in some HIV-infected patients after immune restoration during HAART. Three Japanese patients presenting with diabetes after receipt of HAART have been shown to develop

antibodies to glutamic acid decarboxylase, at a time when CD4 counts shot up suddenly. The type of diabetes associated with HIV may be classified as type 2 diabetes (T2DM), rather than T1DM."

"Concurrent use of opiates among Hiv infected patients may alter beta cell function while heroin addiction is associated with insulin resistance. No specific mechanisms of action have been proposed for these effects."

"HIV infection is linked with hepatitis C infection (HCV), which is associated with insulin resistance and diabetes, due to increased intrahepatic tumour necrosis factor (TNF α) and hepatic steatosis. These factors increase the risk of diabetes in a patient suffering from concurrent HIV and HCV infection. Persons with HCV who are 40 years of age or older are greater than 3 times more likely to have diabetes than those of the same age without HCV infection."

"HIV is also associated with various endocrine abnormalities, including those of the growth hormone axis. These include deficiency of growth hormone, as well as growth hormone resistance. Growth hormone deficiency may contribute to insulin resistance in HIV-infected patients"

"The increased accumulation of visceral fat, with wasting of subcutaneous fat, noted in these patients, creates higher levels of inflammatory cytokines such as TNF α. This in turn leads to diabetes or impaired glucose tolerance by increasing insulin resistance.HIV-infected patients with metabolic syndrome show disturbances in

inflammation and adipokines: they have higher CRP and leptin and lower adiponectin . This may contribute to the pathogenesis of diabetes."

"Anti-retroviral drugs are not the only iatrogenic culprits in HIV-associated diabetes. Drugs used to manage comorbid conditions associated with AIDS may also cause diabetes. Pentamidine, which is used to prevent and treat P. carinii associated pneumonia, can cause β-cell toxicity, with acute hypoglycemia followed by later diabetes .Megesterol acetate, which is used as an appetite stimulant, predisposes to diabetes because of its intrinsic glucocorticoid like activity, increased caloric intake and weight gain . Hypoglycemia has been noted to resolve once megesterol is stopped, and to recur on rechallenging.Patients on HAART may also be predisposed to diabetes because of the improved nutritional status and weight gain that accompanies effective treatment of HIV."

HIV AND CARDIOVASCULAR DISEASE:

Dilated cardiomyopathy

Myocarditis

Autoimmune cardiomyopathy

Nutritional related cardiomyopathy

Pericardial effusion

Endocarditis

Hiv associated pulmonary hypertension

Vasculitis and coronary artery disease

Hypertension

Drug relaed cardiotoxicity

“Hiv disease is an important cause of diated cardiomyopathy with an incidence of about 15.9 in 1000 before HAART.Mortality was higher in patients having depressed left ventricular fractional shortening or increased left ventricular dimension,thickness,mass,wall stress,heart rate,blood pressure.”

HIV AND NEUROLOGICAL DISEASE:

BRAIN (FOCAL)

Cerebral toxoplasmosis

Primary CNS lymphoma

Progressive multifocal leucoencephalopathy

Cryptococoma

Tuberculoma

Varicella zoster encephalitis

NON FOCAL

Hiv associated neurocognitive disorders

Toxoplasmosis

Cytomegalovirus

Herpes encephalitis

Metabolic encephalopathy

Brain abscess

MENINGES:

Aseptic meningitis

Tuberculous meningitis

Syphilitic meningitis

Cryptococcal meningitis

Metastatic meningitis

SPINAL CORD:

Vacuolar myelopathy

Varicella related myelopathy

Metastatic myelopathy

distal sensory polyneuropathy

autonomic Cytomegalovirus myelopathy

Vitamin B 12 related myelopathy

PERIPHERAL NERVES AND NERVE ROOTS:

Acute inflammatory demyelinating polyradiculoneuropathy

chronic inflammatory demyelinating polyradiculoneuropathy

vasculitic neuropathy

brachial plexopathy

lumbosacral plexopathy

cranial mononeuropathy

neuropathy

drugs incuded neuropathy

MUSCLE:

Polymyositis

Pyomyositis

Inclusion body myositis

Zidovudine myopathy

AIDS cachexia myopathy

TREATMENT RELATED IMMUNE RECONSTITUTION INFLAMMMATORY SYNDROME

TB REACTIVATION BY HIV:

"The depletion of $CD4^+$ T cells, which is a main feature of AIDS, is certainly an important contributor to the increased risk of reactivation of latent TB and susceptibility to new M.tuberculosis infection. .Other mechanisms reported to facilitates infection and disease in individuals with HIV are up-regulation of tuberculosis bacteria entry receptors on macrophages , HIV manipulation of macrophage bactericidal pathways , deregulated chemotaxis, and a tipped Th1/Th2 balance. It has also been shown that HIV impairs tumor necrosis factor (TNF)-mediated macrophage apoptotic response toM.tuberculosis and thus facilitates bacterial survival".

“In the latent phase of TB, Several immune mechanisms, such as increased levels of $FoxP3^+$ Treg cells, increased production of IL-27 , TGF-β , PGE-2 ,SOCS1, or the decoy receptor D6, or diminished levels of IFN-γ, TNF, and polyfunctional specific T cells, are believed to play a role in such reactivation. Many of these factors, such as SOCS1 or IL-27, down-regulate the IFN-γ/IL-12 axis, thereby impairing bacterial control, while others, such as the D6 decoy receptor, are mainly anti-inflammatory, but may indirectly inhibit efficient bacterial clearance.”

“Granulomas are organized cellular structures that constitute TB's pathologic hallmark.. $CD4^+$ T cells and TNF are important in maintaining granuloma organization. Granuloma formation may fail in individuals with a compromised immune system, and there are several hypotheses about how HIV exacerbates TB pathology through the manipulation of granulomas”

“Specifically, TB patients with AIDS present a dominant granulocytic infiltrate and necrosis without the typical caseous necrosis seen in non-HIV-infected TB granulomas. This has been associated with the killing of $CD4^+$ cells in the granuloma, probably resulting in a direct disruption of granuloma structure and abolition of the containment of infection. Cavitary lesions are seldom encountered in patients with a CD4 T-lymphocyte count <200/mm^3.As a result, while in the majority of adult patients TB is confined preferentially to the lungs, in HIV-infected patients TB can be a systemic disease involving multiple organs that lack well-defined granulomas and instead develop more diffuse lesions .All forms of extrapulmonary TB have been described in patients with HIVIN”

“While TNF production in response to *M. tuberculosis* infection is required for control of bacterial growth, TNF is known to activate HIV replication in macrophages indicating that the host immune response initiated against one pathogen may promote the replication of another. Thus, both HIV and *M. tuberculosis* stimulate TNF release from infected cells, and TNF hampers bacterial growth while enhancing HIV replication.”

“It has been suggested that TB patients have a microenvironment that facilitates HIV infection by

increasing the expression of co-receptors CXCR4 and CCR5

increasing pro-inflammatory cytokines, especially TNF; and *iii*) down-regulation of CCL5 . It has also been shown M tuberculosis and its cell wall component,

lipoarabinomannan (LAM) may activate replication of HIV in provirus-carrying cells by inducing TNF and IL-6 production through the NF-κB pathway, which in turn triggers transcriptional activation of the long terminal repeat (LTR) promoter and supports replication of HIV."

IMMUNE RECONSTITUTION INLAMMATORY SYNDROME:

"A particularly intriguing phenomenon is immune reconstitution inflammatory syndrome (IRIS). IRIS may develop in M tuberculosis and HIV co-infected patients who undergo anti-TB treatment and antiretroviral therapy (ART) . The patients present with an exacerbation of symptoms and radiological manifestations of TB, and recognized predictors of IRIS include low $CD4^+$ T lymphocyte counts and high plasma viral load prior to initiation of ART, and an increase in $CD4^+$ counts after highly active antiretroviral therapy (HAART) onset. Possible mechanisms responsible for IRIS may be a sustained Th1-response against mycobacterial antigens, which is followed by dysregulation of cytokine secretion and T cell migration to the inflammatory site . Recently, it was shown that patients who developed IRIS had higher pre-ART levels of TNF and increasing levels of inflammation biomarkers.Moreover, it has been demonstrated that TB/HIV co-infected patients who experienced IRIS had significantly lower levels of Abs to the phenolic glycolipid (PGL-TB1) antigen, specific form M tuberculosis compared to patients who did not develop TB-IRIS."

HIV AND CANCER:

20% total death in hiv infected patients were cancer releated

Common cancers in hiv:

Kaposis sarcoma

Non-hodgkins lymphoma

Hodgkin lymphoma

Cervical cancer

CNS lymphoma

Immunoblastic lymphoma

Burkitt’ lymphoma

Anal cancer

Hepatocellular carcinoma

Lung carcinoma

Prostate carcinoma

Oral cancer

Leiomyosarcoma

RISK FACTORS:

Human papilloma virus infection

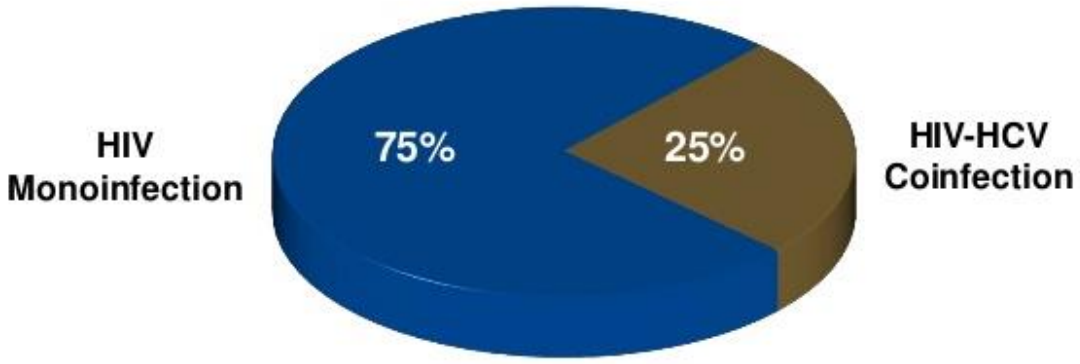
Human herpes virus 8 infection

Ebstein barr virus infection

Hepatitis c coinfection

Smoking

HIV AND HEPATITIS C CO INFECTION:



“The prevalence of hepatitis C in hiv infected patients around 20-30%.the main source of infection is blood borne route through iv needle sharing.following acute hepatitis C infection90% will develop chronic disease.this is significantly higher than the risk of chronicity among those with hepatitis C monoinfection where than 30% will spontaneously clear the virus.”

HIV EFFECT ON CHRONIC HEPATITIS C:

“Hiv infection has been associated with persistent HCV viral load,and reduced response to treatment.hiv virus causes quantitative loss of memory cells which is responsible for elevated HCV RNA level.hiv itself mediates hepatic cytokine release through tat ,gp 120.hiv infection makes hepatic fibrosis much earlier than those who were affected only by HCV.”

EFFECT OF HCV INFECTION ON HIV:

“HIV infection is linked with hepatitis C infection (HCV), which is associated with insulin resistance and diabetes, due to increased intrahepatic tumour necrosis factor (TNF α) and hepatic steatosis. These factors increase the risk of diabetes in a patient suffering from concurrent HIV and HCV infection. Persons with HCV who are 40 years of age or older are greater than 3 times more likely to have diabetes than those of the same age without HCV infection”

“Hiv patients are more prone for fatty liver disease,hepatocellular carcinoma,metabolic syndrome,vasculitic syndromes when they are co infected with hepatitis C virus.”

TOTAL LYMPHOCYTE COUNT

“TLC is easily obtained from the routine blood count with differential through multiplication of lymphocyte percentage by white blood cell count.

TLC= % OF LYMPHOCYTE × TOTAL WBC COUNT

TLC has been suggested as a measure of when to initiate both HAART and prophylaxis against opportunistic illness in resource limited settings. In December 2003, the WHO issued the recommendations for the initiation of ART, when CD4 testing unavailable to include WHO stage 3 or 4 or WHO stage 2 in combination with the TLC<1200 cells/cu.mm “

“CAROLINE COSTELLO, MPH, KENRAD E. NELLSON, MD conducted a study in Thai population on “ Predictors of low CD4 count in resource limited settings”. In this study TLC<1200cells/cu.mm has good corelation with CD4<200cells/µL improved the sensitivity above that of meeting the WHO guidelines for onset of ART while maintaining a specificity above 99%.”

“S.M. ALAVI, F. AHMADI et al conducted a study on “corelation between total lymphocyte counts, haemoglobin, hematocrit and CD4 count in HIV/AIDS patients” in this study a strong correlation was observed between CD4 counts and TLC.”

ANAEMIA

“Disorders of hematopoietic system including lymphadenopathy, anaemia, leucopenia and /or thrombocytopenia are common throughout HIV infections and maybe the direct results of HIV, manifestations of secondary infections, neoplasms or side effects of therapy.”

“Anaemia is most common hematological abnormality in HIV infected patients. Among the specific reversible causes of anaemia in the settings of HIV infections are drug toxicity, systemic fungal and mycobacterial infections, nutritional deficiencies and parvo virus B19 infections. Folate levels are usually normal in HIV infected individuals. Vitamin B12 may be depressed as a result of achlorhydria or malabsorption”.

“True autoimmune anaemia is rare. Erythropoietin levels in patients with HIV infection and anaemia are generally less than expected given the degree of anaemia. Treatment with erythropoietin at doses of 100µg/kg thrice weekly may result in increase in haemoglobin.”

“BELPERIO PS, RHEW DC. Prevalence and outcomes of anaemia in individuals with human immunodeficiency virus. In this study it was found that anaemia was more commonly associated with disease progression with progressive decrease in CD4 count.”

“MOCROFT A, KIRK O, BARTON SE, et al conducted a study on “ anaemia is independent predictive marker for clinical prognosis in HIV infected patients” from across Europe. In this study it was found that anaemia was independent predictor of mortality in HIV infected patients.”

INITIAL EVALUATION OF THE PATIENT WITH HIV INFECTION:

“History and physical examination

Routine chemistry and haematology

Liver function test

Lipid profile test

CD4 T lymphocyte count

Plasma HIV RNA level

HIV resistance testing

HLA-B5701 screening

Rapid plasma regin test& veneral disease research laboratory

Anti toxoplasma antibody titer

Purified protein derivative

Mini mental status examination

Serology test for hepatitis A,B,C.”

INDICATIONS FOR CHANGING ANTI RETRO VIRAL THERAPHY:

Less than a 1 log drop in plasma HIV RNA by 4 weeks following the initation of therapy

A reproducible significant incresase (defined as three fold or greater) from the nadir of plasma HIV RNA level not attributable to intercurrent infection,vaccination,or test methodology

Persistently declining CD4 T cell numbers

Clinical deterioration

Side effects

ANTI RETROVIRAL THERAPY:

NUCLEOSIDE REVERSE TRANSCRITASE INHIBITORS

Abacavir

Didanosine

Emtricitabine

Lamivudine

Stavudine

Zidovudine

NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS:

Tenofovir

NON NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS:

Delavirdine

Efavirenz

Etravirine

Nevirapine

Rilpivirine

PROTEASE INHIBITIRS:

Fosamprenavir

Indinavir

Lopinavir

Nelfinavir

Ritonavir

Saquinavir

Atazanavir

Darunavir

Tipranavir

ENTRY INHIBITORS:

Enfuvirtide

Maraviroc

INTEGRASE INHIBITORS:

Raltegravir

Elvitegravir

Dolutegravir.

ADVERSE EFFECTS OF ANTI RETRO VIRAL THERAPY:

“Zidovudine-anemia,neutropenia,lipoatrophy,lactic acidosis

Abacavir-hypersensitive rash

Didanosine-pancreatitis,neuropathy,fulminant hepatic failure

Stavudine-lactic acidosis,lipoatrophy

Tenofovir-osteoporosis,fanconi syndrome,hypokalemia

Nevirapine-rash ,hepatotoxicity

Efavirenz-rash ,neropsychiatric symtoms

Indinavir-renal stones

Nelfinavir-diarrhea

Atazanavir-gall stones and renal stones

Tipranavir-intracranial bleed

Protease inhibitors-lipodystrophy,diabetes,dyslipidemia.”

AIMS AND OBJECTIVES OF THE STUDY

1.To evaluate the correlation of CD4 counts with the TLC and ALC to suggest that these can be used as a surrogate for CD4 count to initiate ART in resource limited settings.

2. To determine a range of TLC and ALC cut-offs for predicting CD4 count <350 cells/µl, which is important for the initiation of ART.

MATERIALS AND METHODS;

STUDY POPULATION:

The present study is conducted on HIV seropositive patients admitted in any wards or attending outpatient departments of Government Rajaji Hospital, Madurai during the period of Jan2016 to June 2016.

INCLUSION CRITERIA:

The individuals should be above 18 years of age, they should be proven to be HIV-positive and they should not be on prior anti-retroviral therapy (ART).

EXCLUSION CRITERIA:

HIV patients on prior ART

HIV patients who are Pregnant.

Paediatric HIV patients.

HIV Patients on steroid , iron , or vitamin therapy.

HIV Patients with bleeding disorder.

HIV patients with Chronic renal failure patients.

HIV Patients with opportunistic infections or any intercurrent infection likely to alter the lab parameters.

DESIGN OF STUDY:

Cross sectional study

PERIOD OF STUDY:

January2016 To June 2016 (6 months)

COLLABORATING DEPARTMENTS:

ART Centre

Department of Biochemistry

Department of Microbiology.

ETHICAL CLEARANCE:

Necessary ethical clearance was obtained from ethical committee ,GRH , Madurai.

CONSENT: Individual written and informed consent.

ANALYSIS:STATISTICAL ANALYSIS.

CONFLICT OF INTEREST: NIL

FINANCIAL SUPPORT: SELF

PARTICIPANTS:

HIV seropositive patients admitted in any wards or attending outpatient departments of Government Rajaji Hospital, Madurai during the period of Jan 2016 to June 2016.

DATA COLLECTION:

A previously designed proforma is used to collect the demographic and clinical details of the patients. All the patients underwent detailed clinical evaluation, appropriate investigations.

History was taken on details of unprotected sexual intercourse , blood transfusion ,IV drug abuse, repeated respiratory infections,fever , recurrent diarrhoea and unexplained weight loss. Presence of lymphadenopathy, oral ulcers, splenomegaly and peripheral neuropathy will be noted. Hemoglobin, ,complete blood count including total WBC count and differential count , blood urea, serum creatinine, blood glucose, liver function tests including serumbilirubin, serum transaminases, will beestimated. CD4 count , Total lymphocyte count and absolute lymphocyte count are also estimated .The study group was divided on the basis of CD4 count

into 200 to 350 cells/cu.mm and to <200 cells/cu.mm. various cut offs for absolute lymphocyte count and total lymphocyte count was studied in these groups. And a range of absolute lymphocyte count and total lymphocyte count cut offs for CD4 count <350 cells/cu.mm was determined which can be used to initiate antiretroviral therapy in resource limited settings.

LABORATORY INVESTIGATIONS:

The following specific investigations were done

1.Hemoglobin

2.Complete blood count including total WBC count and differential count

3.CD4 count

4. absolute lymphocyte count and total lymphocyte count.

CD4 COUNT :

CD4 count was done using flowcytometry . flow cytometers use lasers to excite fluorescent antibody probes specific for various cell surface markers , such as CD3, CD4 , and CD8. It is calculated in cells/µL.

AUTOMATED CELL COUNTER

Total WBC count , Differential count , haemoglobin ,were obtained using automated cell counter.

Automated cell counters sample the blood , and quantify and describe the cell populations using both electrical and optical techniques.

Total lymphocyte count was easily obtained from total WBC count and differential percentage of lymphocyte using the following formula

TLC=% of lymphocyte × total WBC count.

Normal TLC is usually >1800 cells/cu.mm.

LIMITATIONS OF OUR STUDY

1.WHO clinical staging were done on presumed clinical basis and some basic laboratory investigations . so , some patients may be wrongly staged.

2.Small sample size of the study.

3.Asymptomatic HIV patients with opportunistic infections could not be identified and could have masked the alterations in total lymphocyte count as well as CD4 count.

STATISTICAL TOOLS

The information collected regarding all the selected cases were recorded in a master chart. Data analysis was done with the help of computer using epidemiological information package.

Using this software range, frequencies , percentage , mean , standard deviations , chi square and ‘p’ values were calculated. Kruskul wallis chi square test was used to test the significance of difference between quantitative variables and Yate’s test for qualitative variables. A ‘P’ value less than 0.05 denotes significant relationship.

RESULTS

The study population includes 100 patients.

TABLE 1 : AGE DISTRIBUTION

AGE GROUP	NO.OF CASES	%
≤20	3	3
21-30	20	20
31-40	54	54
41 - 50	17	17
> 50	6	6
Total	100	100

In our study about 54% of patients were in age group between 31-40 years . 91% of patients are in age group between 21-50 years. So majority of patients in our study were in reproductive age group.

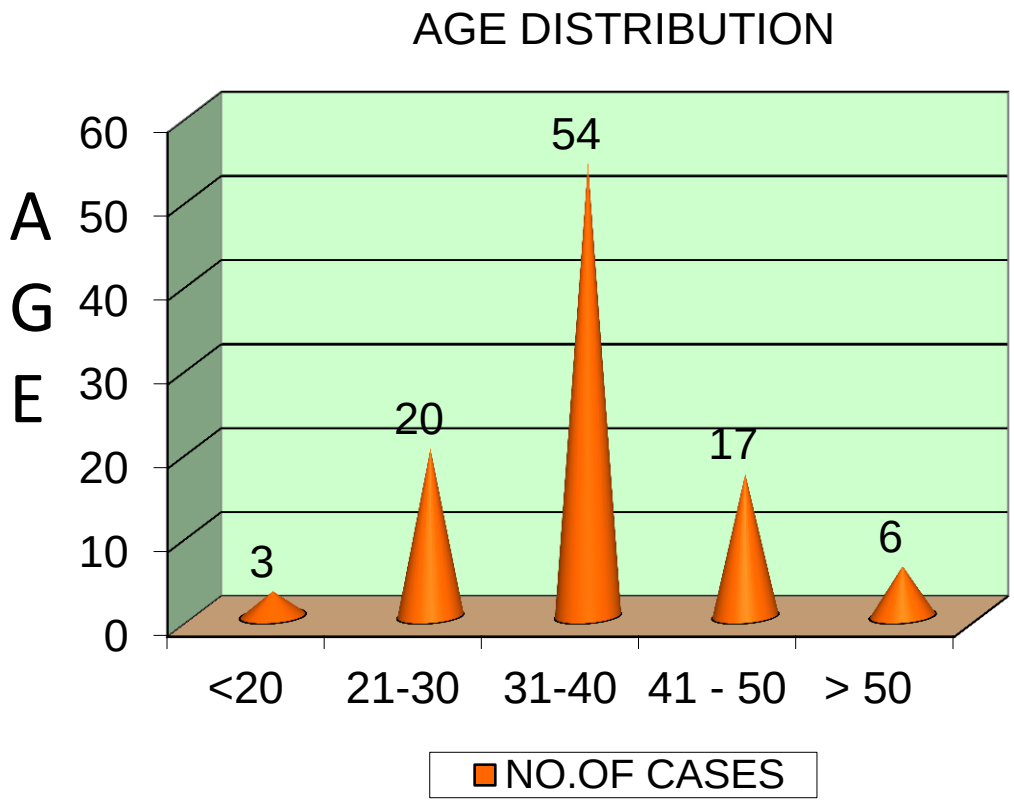


TABLE 2 : GENDER DISTRIBUTION

SEX	NO.OF CASES
MALE	67
FEMALE	33
Total	100

In our study about 67% of patients were males and 33 % were females.

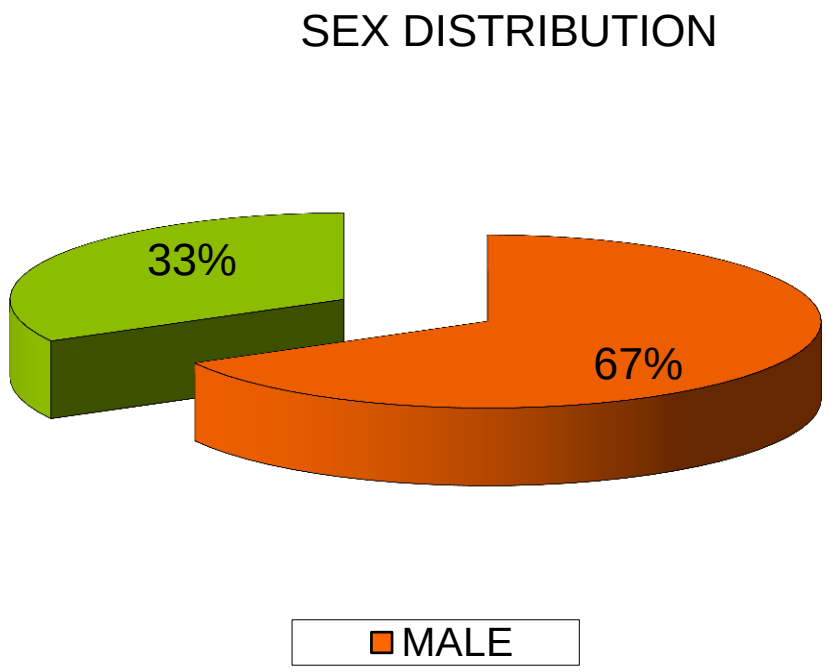


TABLE 3 : EXPOSURE TYPE:

RISK FACTOR	NO.OF CASES
Heterosexual	83
Homosexual	12
Unknown	5
Total	100

The above table shows that the heterosexual mode of transmission was the commonest mode of transmission accounting for 83 % of the cases. Around 12 % of cases transmission was through homosexual route esp(male to male transmission).

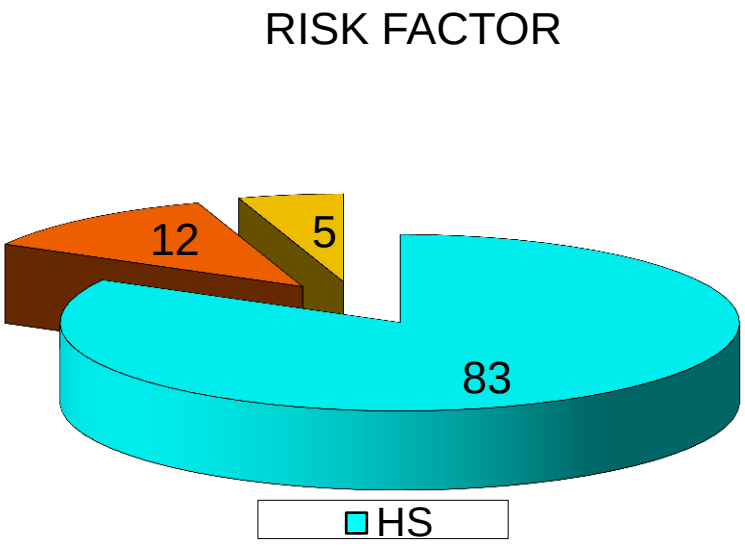


TABLE 4: CLINICAL STAGING AND NO. OF CASES

CLINICAL STAGING	NO.OF CASES
I	29
II	31
III	18
IV	22
Total	100

As shown in the above table , about 60% of the patients came under WHO stage 1 and 2 and 40% came under WHO stage 3 and 4.

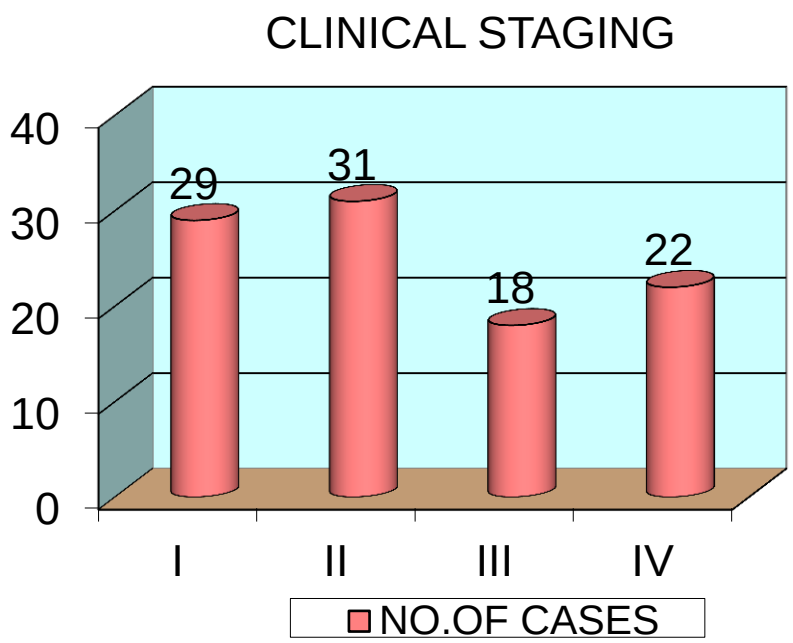


TABLE 5: DISTRIBUTION OF CD 4 COUNT

CD4 COUNT	NO.OF CASES
< 100	6
101 - 200	23
201 - 350	52
> 350	19
Total	100

The CD 4 counts were grouped under four different headings. They are <100 cells/µl, 101-200 cells/µl, 201-350 cells/µl and >350 cells/µl.

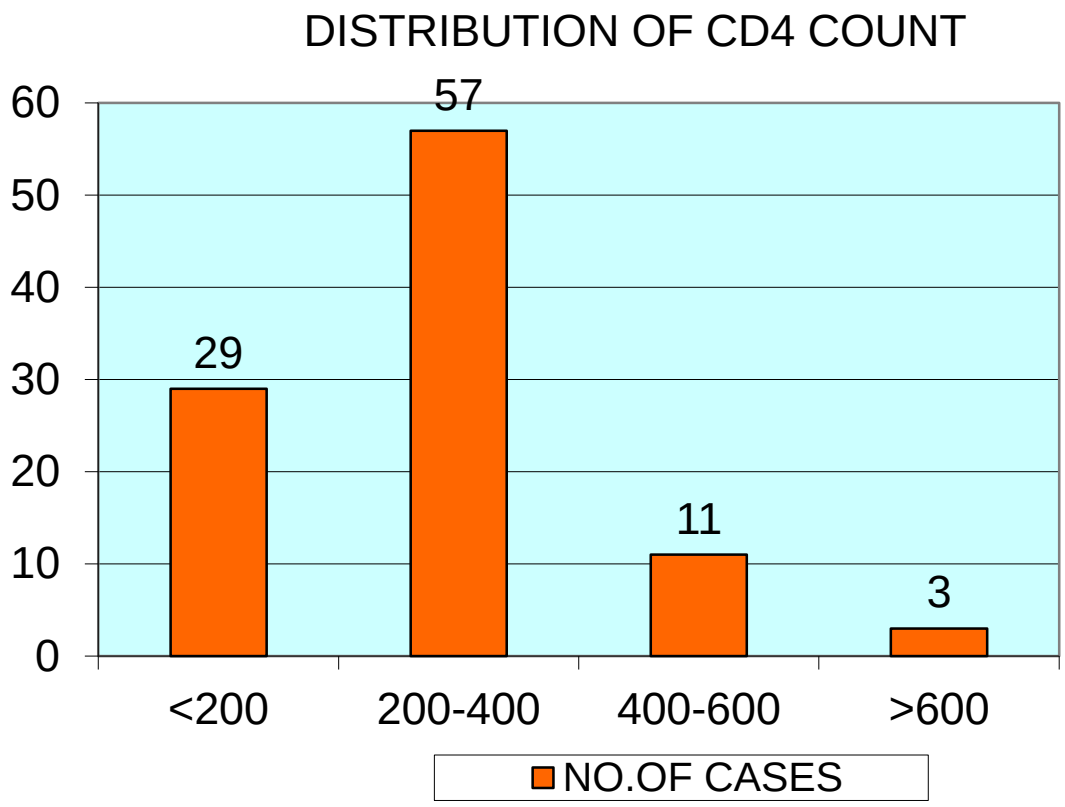


TABLE 6 : CD4 COUNT AND CLINICAL STAGING:

CLINICAL STAGING	MEAN	SD
I	381.28	148.49
II	243.87	95.91
III	281.72	98.24
IV	155.86	69.77

Stage 4 had the lowest mean CD4 count (155.86/µL) among all stages. Stage 1 had the highest mean CD4 count (381.28/µL) when compared to all stages. Stage 2 had mean CD4 count of 243.87/µL and stage 3 had CD4 count of 281.72/µL.

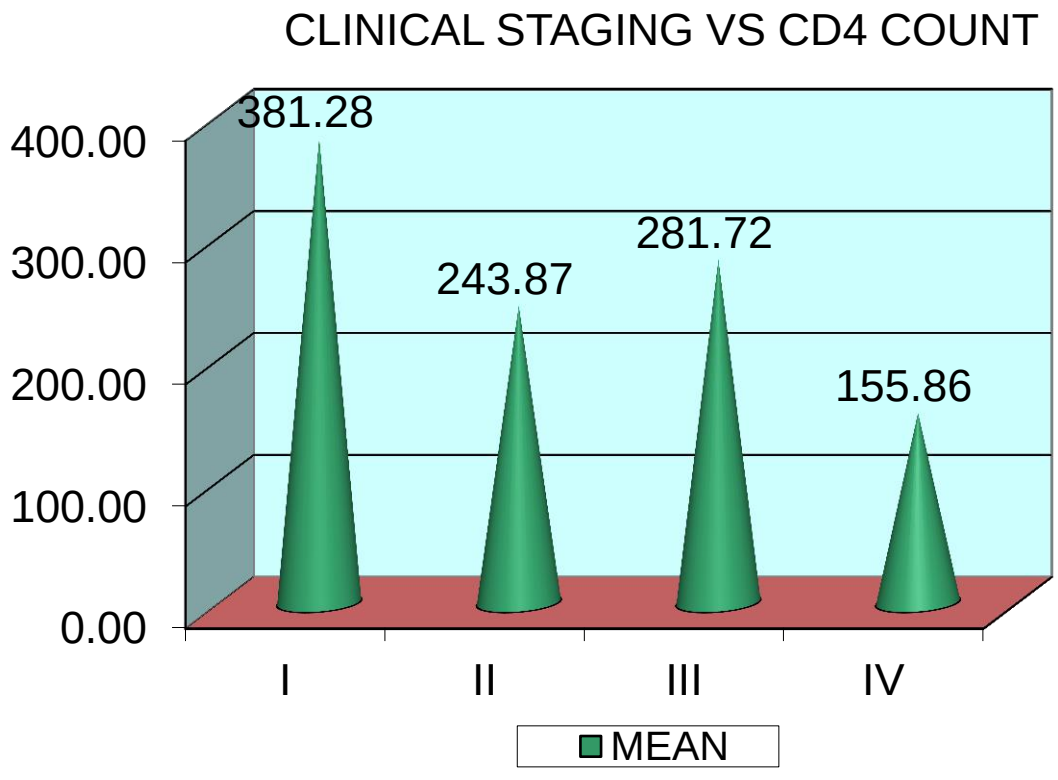


TABLE TOTAL LYMPHOCYTE COUNT(TLC) AND CLINICAL STAGING

CLINICAL STAGING	MEAN	SD	
I	1809	363.29	
II	1613.83	331.76	<0.001
III	1584.03	301.72	
IV	1275.96	284.06	

Stage 4 had lowest mean TLC of 1275.96 cells/cumm. Stage 1 had the highest mean TLC of 1809 cells. Stage 2 had mean TLC of 1613.83 cells and stage 3 had mean TLC of 1584.03 cells.

TABLE HEMOGLOBIN AND CLINICAL STAGING:

CLINICAL STAGING	MEAN	SD	
I	9.82	1.04	
II	7.34	1.39	<0.001
III	6.79	1.34	
IV	5.29	1.12	

Stage 4 had lowest mean haemoglobin of 5.29g and stage 1 had highest mean haemoglobin of 9.82 gm . stage 2 had mean haemoglobin of 7.34gm and stage 3 had mean haemoglobin of 6.79gm.

TABLE ABSOLUTE LYMPHOCYTE COUNT AND CLINICAL STAGING

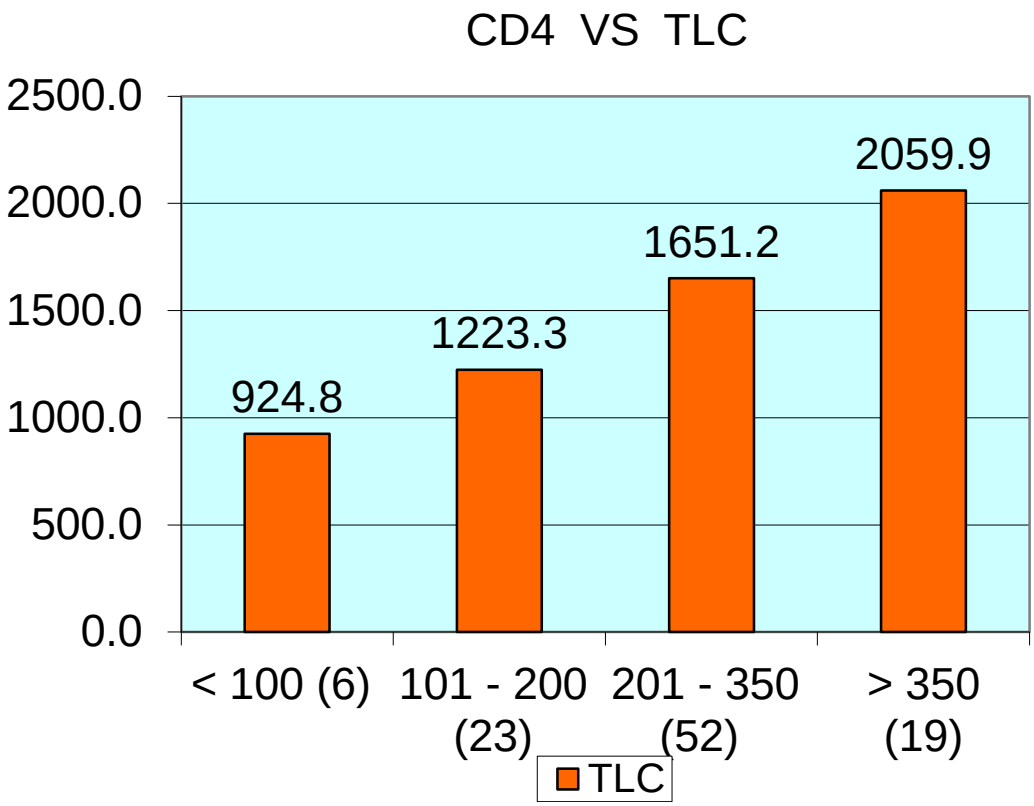
CLINICAL STAGING	MEAN	SD	
I	1284.03	120.48	
II	1226.04	147.99	<0.001
III	1196.11	177.05	
IV	1095.00	142.79	

The mean absolute lymphocyte count in stage 4 was 1095 cells , in stage 1 was 1284 cells , in stage 2 was 1196.4 cells and in stage 3 was 1196.45 cells.

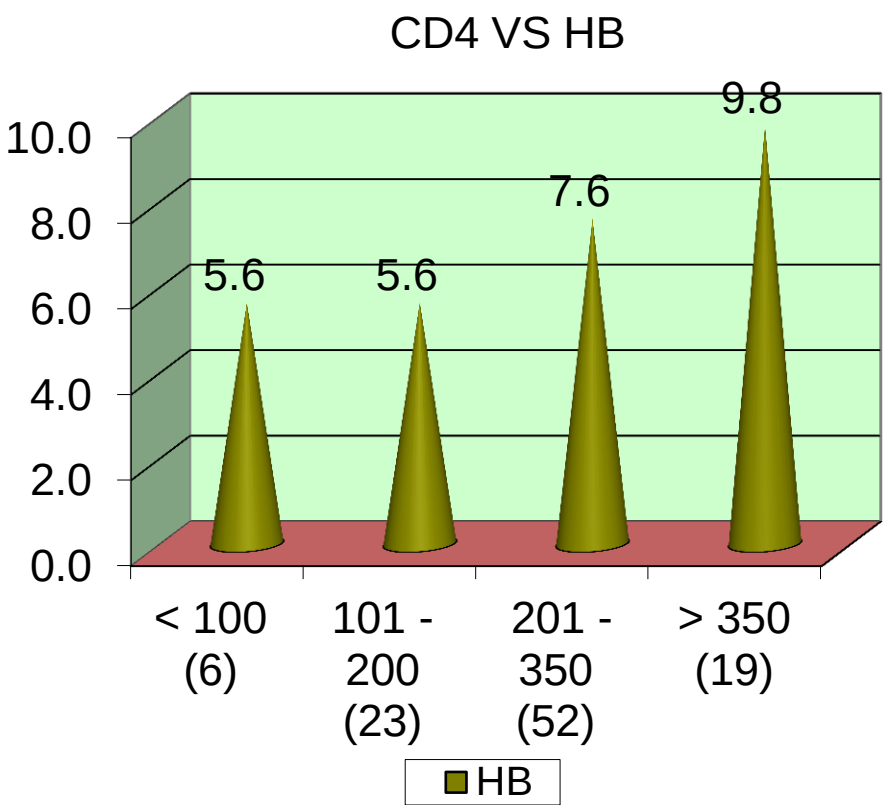
TABLE MEAN PATTERN OF STUDY PARAMETERS WITH CD4 COUNTS

	TLC		HB		ALC	
	Mean	SD	Mean	SD	Mean	SD
< 100 (6)	924.833	106.925	5.633	1.46	904.833	157.093
101 - 200 (23)	1223.261	208.645	5.639	1.159	1052.696	96.303
201 - 350 (52)	1651.212	150.683	7.59	1.647	1242.154	91.271
> 350 (19)	2059.947	241.399	9.774	1.634	1353.368	94.004
P VALUE	< 0.001	SIG	< 0.001	SIG	< 0.001	SIG

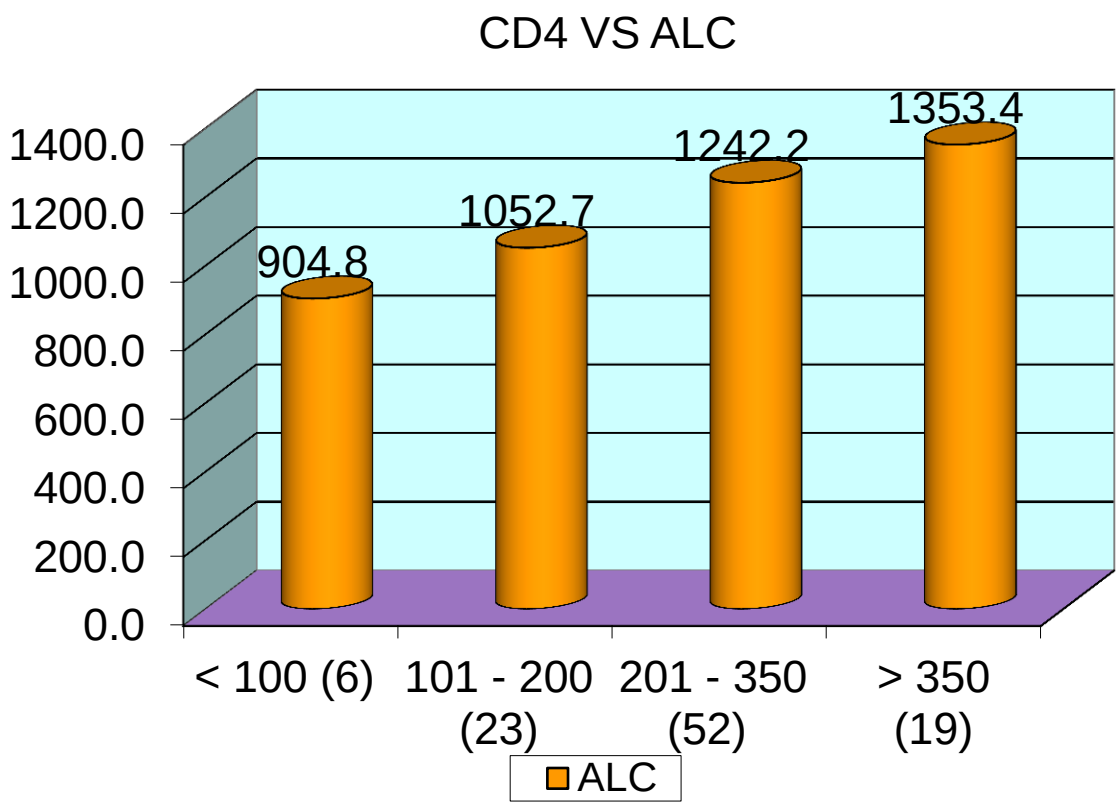
In our study the Total Lymphocyte Count, absolute lymphocyte count and hemoglobin showed an upward trend with CD 4 count.



The mean TLC for CD4 count less than 350 cells/µL is 1266.4 cells/cu.mm. and mean TLC for CD4 cont less than 200 cells/µL is 1074.05 cells/cu.mm.



The mean Hemoglobin for CD4 count <350 cells/µL is 6.26 gm and the mean haemoglobin for CD4 count <200 cells/µL is 5.6 gm



The mean ALC for CD4 count <350 cells/µL is 1066.56 cells/cu.mm. and the mean ALC for CD4 count <200 cells/µL is 978.75 cells/cu.mm.

DISCUSSION:

"If CD4 count is available and HIV infected patient's CD4 lymphocyte count is less than 200 cells/µL , it would be universally agreed that ART should be initiated regardless of the fact that CD4 counts have biologic variability by sex , age , and ethinicity as well as some variability in measurement in the laboratory."

"Although the CD4 count has become the gold standard but in resource limited seetings where the CD4 count is not routinely available , other clinical and laboratory

parameters can be predictive of severe immune compromise. Routine hemotologic testing is inexpensive and widely accessible and can provide measurements of the TLC and haemoglobin level."

"In our study about 100 patients are randomly enrolled , of which 67% are males and 33% are females. Most of the patients are in the reproductive age group. 74% of the patients are in the age group between 21-40 years. This shows that HIV is more in reproductive age group. This indicates a trend of young and productive generation being affected; a reflection of the devastating effects india will face as the younger generation work force is affected."

In this study age and type of exposure are also studied and correlated with CD4 count . "The most common mode of transmission is heterosexual route (83%). This is in consistent with UZAGARE R et al who found that the percentage of various mode of transmission as sexual route (93%) commonly heterosexual , blood (2.32%) , perinatal(2.32%) , surgery and IV needles (3.72%). This is also similar to the findings in studies by A.R.Sircar et al (64% heterosexual), A.D Harris (62% heterosexual) and Kothari et al (68%heterosexual)."

STAGING:

WHO in 2005 proposed a revised clinical staging to initiate ART in resource limited settings especially in Africa and Asia.

CLINICAL AND IMMUNOLOGICAL CRITERIA FOR INITIATING ART IN ADULTS AND ADOLESCENTS

CLINICAL STAGE	ART
STAGE 4	Treat
STAGE 3	Consider treatment CD4 if available can guide the urgency with which ART should be started
STAGE 1 , 2	Only if CD4 <350cells/µL

"Many studies were conducted to asses the WHO recommendation for clinical criteria to initiate ART in resource limited settings."

"In our study using WHO clinical staging 4 when correlated with CD4 count < 200 cells/µL we get a significant statistical p value. Patients with stage 4 had mean CD4 count 155.86 cells/µL and patients with CD4 count < 200 cells/µL were in stage 3 or stage 4(93%)"

Comparison of distribution according to CD 4 counts in different studies

CD4 counts	**Our study (n=50)**	**Beck (n=153)**	**John Hopkins study (n=5864)**	**YRG study (n=1315)**
<100	**6(6.0%)**	**12 (13%)**	**1055(18%)**	**105(8%)**
101-200	**23(23.0%)**	**53 (34%)**	**1584(27%)**	**395(30%)**
201-350	**52(52.0%)**	**78 (53%)**	**3225(55%)**	**905(62%)**

TOTAL LYMPHOCYTE COUNT

"In December 2003 , the WHO broadened the recommendations for initiation of ART when CD4 testing is unavailable. Many studies have evaluated the use of TLC as a surrogate marker for CD4 cell count with mixed results. In our study the mean TLC for CD4 count less than 350 cells/µL is 1266.4 cells/cu.mm. and mean TLC for CD4 cont less than 200 cells/µL is 1074.05 cells/cu.mm. CAROLINE COSTELLO, MPH , KENRAD E. NELSON , MD , conducted a study on predictors of low CD4 count in resource limited settings. In this study TLC<1200 cells/cu.mm correlated with CD4 count <200 cells/µL. In a study "complete blood count as a surrogate CD4 cell marker for HIV monitoring in resource limited settings" by RAY Y. CHEN , MD , ANDREW O. WESTFALL , MSJ . MICHAEL HARDIN also showed correlation of TLC <1200 cells/cu.mm to CD4 count <200 cells/µL."

"In our study TLC<1100 cells/cu.mm correlates with CD4 count <200 cells/µL and TLC < 1300 cells/ cu.mm correlates with CD4 count <350 cells /µL. As CD4 count <350 cells/mm^3 is used as a cut off for anti-retroviral therapy, we further evaluated for a correlation between TLC and CD4 count <350 cell/mm^3 and found a correlation between TLC < 1300 for CD4 count <350 cells/µL. Similar higher TLC cutoff values have been used in studies by Jacobson *et al*.where he used a TLC <1900 cells/mm^3 as cut off to predict CD4 count <350cells/mm^3. Kumaraswamy and his colleagues observed that with a TLC, <1400 cells/mm^3, 73% of patients with CD4 cell counts <200 cells/mm^3 were identified. With a TLC <1700 cells/mm^3, 70% of patients with a CD4 cell count of <350 cells/mm^3, requiring initiation of therapy for opportunistic

infection, were identified. In contrast, some studies have shown TLC to be an imperfect predictor of CD4 count."

"This decrease in CD4 count is directly reflected in total lymphocyte count . measurement of peripheral blood CD4 T lymphocyte is probably the most important laboratory assay for evaluation and monitoring of patients with HIV. The most common technique for measuring CD4 count in developed country setting is flow cytometry which uses lasers to excite fluroscent antibody probes specific for various cell surface markers such as CD3, CD4, CD8 , which distinguish one type of lymphocyte from other."

ANEMIA:

"Anemia is more common in India . in our study 82% of the patients are anemic. 92% are females and 77% are males were anemic. Patients with Hb <5g% were having mean CD4 count of 149 cells/µL. Anemia very well correlated with CD4 count with a stastically significant p value".

"BELPERIO PS, RHEW DC Prevalance and outcomes of anemia in individuals with human immunodeficiency virus. In this study it was anemia more commonly associated with the disease progression with progressive decline in CD4 count and anemia was more common in females when compared to males."

"CAROLINE COSTELLO , MPH , KENRAD E.NELSON,MD , Predictors of low CD4 count in resource limited settings . in this study also anemia was more common

among females than males.When anemia combined with TLC the accuracy of the test is increased.”

ABSOLUTE LYMPHOCYTE COUNT:

“Studies done prior to 2005, showed a relationship between the declines in ALC and CD4 lymphocyte counts as a strong predictor for short term disease progression. Thus during the era of scarce availability of testing laboratories as well as prohibitive cost of CD4 testing, ALC became an acceptable surrogate marker of immune function. Two recent studies one by **Nyawira GIthinji** et al and other by **Sreenivasan** et al have again highlighted the existence of a relationship between ALC and CD4 lymphocyte counts. Even these two studies reveal a weak-modest relationship between these two markers and definitely no capability of detecting immunological and virological failures likely to contribute of ART resistance. A similar study by **Sen** S et al, in 2011 did not validate ALC as an effective surrogate marker of CD 4 lymphocyte counts”.

“In our study, a positive ALC change is shown to be a sensitive and specific marker of positive CD4 change. In our study , The mean ALC for CD4 count <350 cells/µL is 1066.56 cells/cu.mm. and the mean ALC for CD4 count <200 cells/µL is 978.75 cells/cu.mm.”

CONCLUSION:

“1.Majority of patients in our study were in reproductive age group. This indicates a trend of young and productive generation being affected; a reflection of the devastating effects India will face as the younger generation work force is affected.”

2.Most common mode of HIV transmission was heterosexual. Also there is a rise in transmission through male to male sexual contact.

“3. In our study TLC<1100 cells/cu.mm correlates with CD4 count <200 cells/µL and TLC < 1300 cells/ cu.mm correlates with CD4 count <350 cells /µL”

“4. In our study using WHO clinical staging 4 when correlated with CD4 count < 200 cells/µL we get a significant statistical p value. Patients with stage 4 had mean CD4 count 155.86 cells/µL and patients with CD4 count < 200 cells/µL were in stage 3 or stage 4(93%).”

“5. The mean Hemoglobin for CD4 count <350 cells/µL is 6.26 gm% and the mean haemoglobin for CD4 count <200 cells/µL is 5.6 gm%.”

“6. , The mean ALC for CD4 count <350 cells/µL is 1066.56 cells/cu.mm. and the mean ALC for CD4 count <200 cells/µL is 978.75 cells/cu.mm.”

SUMMARY

“This study “ Utility of total lymphocyte count and absolute lymphocyte count as a surrogate marker for CD4 count to initiate ART” was conducted in 100 HIV positive patients who attended ART medical centre in Government Rajaji Hospital , Madurai from January 2016 to June 2016”.

“In our study 100 patients were randomly selected of which 67% were males and 33% were females. Above 74% were in age group between 21 to 40 years showed a prevalence of disease in reproductive age group.”

“In all the patients various clinical and inexpensive laboratory measures such as WHO clinical staging , Total lymphocyte count , Hemoglobin , Absolute lymphocyte count were done and correlated with CD4 count. It was analysed that these parameters can used as a surrogate marker for CD4 count to initiate HAART.”

“In our study TLC<1100 cells/cu.mm correlates with CD4 count <200 cells/µL and TLC < 1300 cells/ cu.mm correlates with CD4 count <350 cells /µL.

The mean Hemoglobin for CD4 count <350 cells/µL is 6.26 gm% and the mean haemoglobin for CD4 count <200 cells/µL is 5.6 gm%

The mean ALC for CD4 count <350 cells/µL is 1066.56 cells/cu.mm. and the mean ALC for CD4 count <200 cells/µL is 978.75 cells/cu.mm.”

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PROFORMA

Name:

Age / Sex:

IP / OP no:

Occupation:

Presenting complaints:

H/o unexplained weight loss.

H/o diarrhoea

H/o fever for >1 month

Past History:

H/o , unprotected sexual intercourse, blood transfusion, tattoing, iv drug use,

H/o CLD, DM, HT, CKD, CVD, DRUG INTAKE, THYROID DISORDERS,EPILEPSY,HEPATITIS.

Personal history

alcoholic/ non alcoholic

smoker/ nonsmoker

Clinical Examination:

General examination	Nutrition Consciousness Orientation Afebrile/Febrile Pallor/no pallor Cyanosis/ No cyanosis Clubbing/No clubbing Pedal edema / no pedal edema
Vitals	Height Weight Temperature Pulse rate Blood pressure Respiratory rate Oxygen saturation Waist , Hip circumferences Body mass index (BMI)
Eyes	
Ear / Nose	
Oral cavity **Endocrine : thyroid**	

Lymph node **Characteristics:** ∘ Size (two dimensions, in millimeters) ∘ Consistency (hard, fluctuant, soft) ∘ Tenderness ∘ Mobility ∘ Definition (discrete, matted) ∘ Symmetry	**Node Sites:** ▪ Posterior cervical chain ▪ Anterior cervical chain ▪ Submandibular ▪ Supraclavicular ▪ Submental ▪ Axillary ▪ Epitrochlear ▪ Inguinal ▪ Femoral
Lungs	
Heart	
Breast	
Abdomen	
Genitals / Rectum **Extremities / Musculoskeletal**	
Skin	

CNS	
Psychiatric	

Laboratory investigations:

1.Hemoglobin,complete blood count.

2.CD4 count

3.Total lymphocyte count (TLC)

4. Absolute lymphocyte count (ALC).

Diagnosis

LIST OF ABBREVIATIONS:

HIV: Human immunodeficiency virus.

AIDS: Acquired immune deficiency syndrome.

CD4: Cluster differentiation 4

TLC: Total lymphocyte count

ALC: Absolute lymphocyte count.

Hb: Hemoglobin.

WHO: World health organisation.

ART: Anti Retroviral therapy.

PCR: Polymerised chain reaction.

EIA : Enzyme immune assay.

MASTER CHART

ART NO.	AGE	SEX	RISKFACTOR	CLINICALSTAGING	CD4COUNT	TLC	Hb	ALC
865/16	48	M	HS	III	100	989	5.6	876
866/16	38	M	HS	III	98	809	7.8	788
867/16	49	M	HS	II	187	1487	6	877
868/16	32	M	HS	II	310	1678	8	1200
869/16	56	M	HS	IV	224	1189	4.5	1221
870/16	36	M	HS	I	388	1689	10	1121
871/16	32	F	HS	III	234	1800	5.5	1109
872/16	54	M	MSM	I	345	1765	9.8	1278
873/16	35	M	HS	IV	55	767	4.5	677
874/16	32	M	HS	IV	244	1654	4.6	1123
875/16	32	M	MSM	III	296	1734	7.9	1109
876/16	54	M	HS	I	137	1432	8	990
877/16	32	F	U	II	162	1324	6.5	1009
878/16	23	M	MSM	II	414	2455	8.7	1433
879/16	39	M	HS	II	212	1675	7.6	1143
880/16	39	F	HS	I	650	2234	11	1433
881/16	28	M	HS	IV	125	1265	3.3	1006
882/16	36	M	U	II	250	1650	6.6	1232
883/16	39	F	HS	I	620	2245	10	1311
884/16	34	M	HS	IV	208	1762	5.6	1208
885/16	36	F	HS	III	345	1609	7.9	1205
886/16	28	M	MSM	II	321	1709	8	1250
887/16	36	M	MSM	II	311	1804	8	1365
888/16	34	M	HS	I	342	1245	9	1298
889/16	25	M	U	I	303	1654	9.3	1309
890/16	34	M	HS	I	243	1609	9.9	1324
891/16	54	F	HS	III	284	1367	6.5	1365
892/16	33	M	HS	II	234	1734	7	1321
893/16	45	M	HS	I	213	1221	9	1114
894/16	34	M	HS	II	112	1009	4.5	1076
895/16	36	F	HS	I	543	2431	11	1344
896/16	36	M	MSM	II	487	2211	8	1333
897/16	38	M	HS	I	564	2110	11	1321
898/16	35	M	U	III	323	1788	7.8	1343
899/16	26	M	HS	II	308	1877	7.5	1376
900/16	19	F	HS	IV	126	1265	6.6	1007
901/16	35	M	HS	III	254	1564	6.6	1310
902/16	36	F	HS	I	223	1565	8	1321
903/16	26	M	U	II	256	1655	7.6	1231

904/16	38	M	HS	I	206	1676	8.8	1211
905/16	23	M	U	I	306	1876	8.9	1309
906/16	34	M	HS	IV	122	1345	6.3	1034
907/16	36	F	U	III	317	1834	8.9	1387
908/16	19	F	HS	II	114	1309	5.5	1012
909/16	60	M	U	II	187	1323	4	1003
910/16	36	F	HS	II	212	1678	6.5	1176
911/16	33	M	U	IV	101	998	5.6	1004
912/16	31	F	HS	I	234	1675	9	1212
913/16	33	M	MSM	II	254	1609	4.5	1221
914/16	28	M	MSM	II	234	1603	5	1208
915/16	36	M	HS	II	208	1608	6	1200
916/16	36	M	HS	III	267	1645	5.3	1278
917/16	37	M	HS	IV	199	1342	5.6	965
918/16	37	M	HS	II	176	1119	6.4	978
919/16	43	M	HS	I	456	2187	10	1453
920/16	46	F	HS	I	234	1678	8	1143
921/16	24	F	HS	IV	218	1652	5.3	1100
922/16	45	M	MSM	III	122	1023	7.5	1000
923/16	27	F	U	II	133	1008	4.6	1008
924/16	35	M	HS	II	234	1609	8	1342
925/16	52	M	HS	I	342	1603	9	1456
926/16	38	M	HS	IV	98	998	4.5	1113
927/16	42	F	HS	I	654	2075	11	1432
928/16	48	M	U	III	299	1699	6	1232
929/16	36	F	HS	II	209	1655	8	1276
930/16	44	M	MSM	I	348	1677	11	1290
931/16	37	M	HS	IV	111	1000	4.7	1099
932/16	24	M	HS	I	332	1654	10	1132
933/16	43	M	HS	III	254	1632	6.8	1100
934/16	39	M	HS	I	234	1678	10.8	1109
935/16	29	F	U	II	338	1654	8.9	1387
936/16	28	M	HS	II	388	1876	6.7	1354
937/16	35	F	HS	IV	129	1611	4	1211
938/16	45	M	MSM	III	432	2167	9.8	1309
939/16	38	M	U	IV	211	1342	4.8	1123
940/16	39	M	HS	I	324	1678	9	1243
941/16	46	M	HS	II	222	1698	6.6	1287
942/16	42	F	HS	IV	131	1232	5.4	1208
943/16	50	M	HS	II	166	1709	5.6	1009
944/16	34	F	U	III	345	2000	6.7	1299
945/16	26	F	HS	IV	94	999	4.4	987
946/16	32	M	HS	I	544	2156	11.3	1389
947/16	33	M	HS	I	578	2099	11.6	1420

948/16	48	F	HS	II	343	1690	6.7	1287
949/16	26	M	HS	I	541	2019	11	1243
950/16	28	F	HS	IV	117	1200	4.5	1117
951/16	42	M	HS	III	322	1655	7.8	1223
952/16	35	M	HS	IV	356	1666	5.2	1432
953/16	18	F	U	IV	120	1002	6.6	1223
954/16	26	F	U	I	442	2111	9.9	1455
955/16	32	F	HS	I	324	1765	9.6	1342
956/16	39	F	HS	II	367	1698	9.7	1243
957/16	24	F	HS	IV	111	1008	6	1111
958/16	48	M	HS	III	456	2056	9.8	1454
959/16	36	M	U	III	323	1678	8	1143
960/16	29	M	HS	IV	222	1654	8.6	1121
961/16	35	F	HS	II	111	1004	6.7	1265
962/16	36	M	MSM	I	387	1654	10	1234
963/16	37	F	HS	IV	107	1120	5.8	1000
964/16	24	F	U	II	100	987	7	988

TLC: Total lymphocyte count

ALC: Absolute lymphocyte count.

Hb: Hemoglobin

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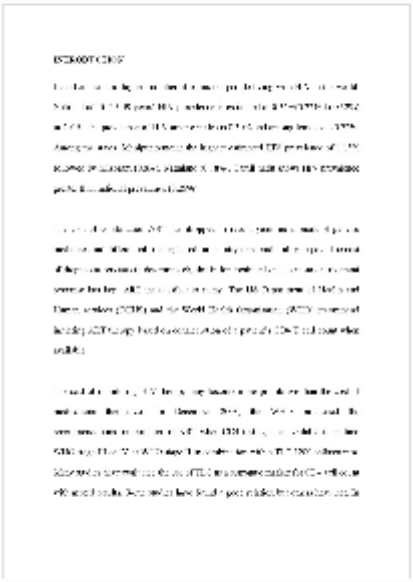

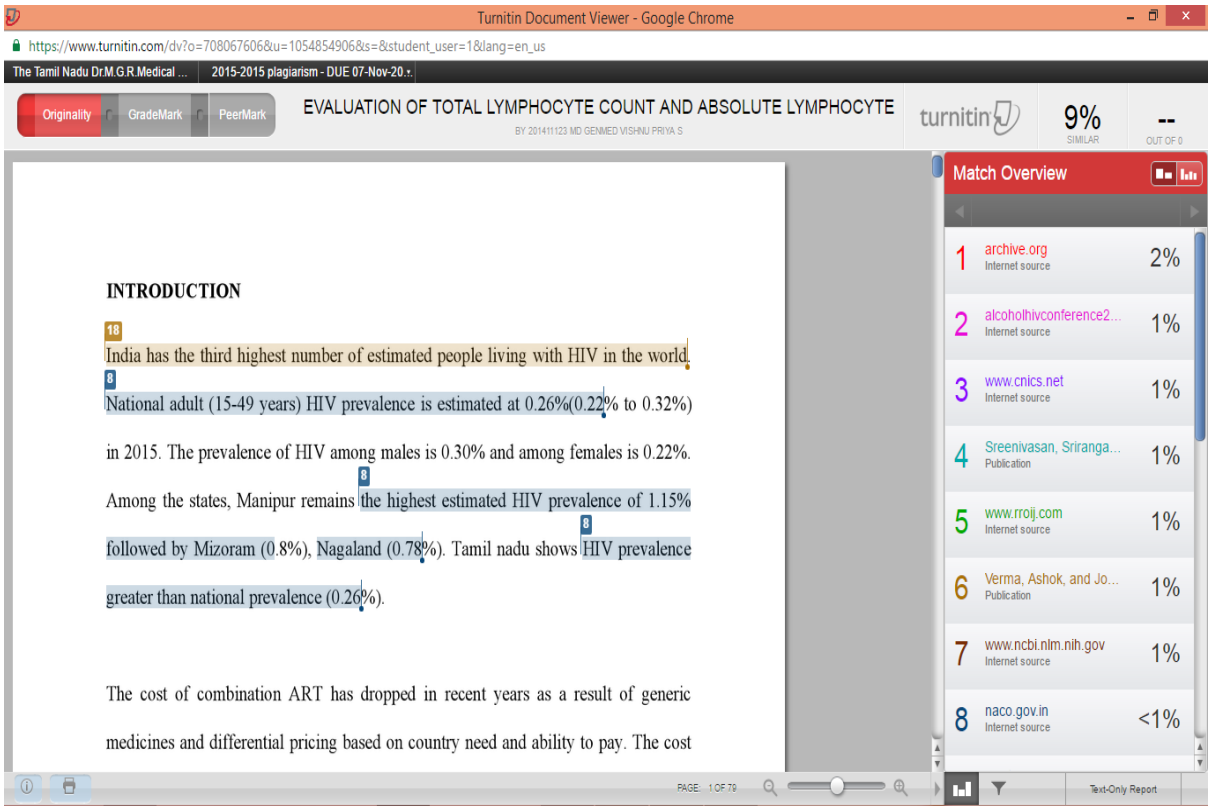
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INTRODUCTION
India has the third highest number of estimated people living with HIV in the world.
National adult (15-49 years) HIV prevalence is estimated at 0.26%(0.22% to 0.32%) in 2015. The prevalence of HIV among males is 0.30% and among females is 0.22%. Among the states, Manipur remains the highest estimated HIV prevalence of 1.15% followed by Mizoram (0.8%), Nagaland (0.78%). Tamil nadu shows HIV prevalence greater than national prevalence (0.26%).
The cost of combination ART has dropped in recent years as a result of generic medicines and differential pricing based on country need and ability to pay. The cost
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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.S.Vishnu priya
Course	:	PG in MD, General Medicine
Period of Study	:	2014-2017
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Evaluation of Total lymphocyte count and absolute lymphocyte as a CD4 T cell surrogate to initiate antiretroviral therapy
Ethical Committee as on	:	16.03.2016

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary Chairman Dean / Convenor

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18 MAR 2016