A REAL-TIME PCR BASED STUDY ON HIV VIRAL LOAD AND ITS CORRELATION WITH THE CLINICAL AND IMMUNOLOGICAL STATUS OF THE HIV POSITIVE PATIENTS

**Dissertation Submitted in
fulfillment of the University regulations for**

**MD DEGREE IN
DERMATOLOGY, VENEREOLOGY AND LEPROSY
(BRANCH XII A)**

MADRAS MEDICAL COLLEGE

**THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI
APRIL 2012**

CERTIFICATE

Certified that this dissertation titled **"A REAL-TIME PCR BASED STUDY ON HIV VIRAL LOAD AND ITS CORRELATION WITH THE CLINICAL AND IMMUNOLOGICAL STATUS OF THE HIV POSITIVE PATIENTS"** is a bonafide work done by **Dr. G.RAJESH KUMAR,** Post graduate student of the Department of Dermatology, Venereology and Leprosy, Madras Medical College, Chennai – 3, during the academic year 2009 – 2012. This work has not previously formed the basis for the award of any degree.

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SPECIAL ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

Constraint of space may have led to few names gone missing in my acknowledgement. I wish to express sincere gratitude to all those who have been associated with my study and have helped me in bringing this dissertation to the present form.

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Introduction

<u>INTRODUCTION</u>

HIV infection/AIDS has become a major public health problem in India. Although the median interval between HIV-1 infection and the development of the Acquired Immune Deficiency Syndrome in adults is 10 to 11 years, some infected persons rapidly progress to AIDS in less than five years. The biological basis of this variability is unknown but differences in viral strains, host immune response and exposure to microbial or environmental factors probably contribute. Identification of laboratory tests that help predict the progression to AIDS in people infected with HIV is desirable because of its implication for both clinical management and prognosis.

Quantitative measurement of HIV RNA levels in peripheral blood has contributed significantly to the understanding of the pathogenesis of HIV infection and has been shown to be an essential parameter in the prognosis and optimal management of HIV infected individuals . CD4+ T cell count is the laboratory test generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection.It has been shown to correlate very well with the level of immunologic competence of the patient.

In view of above, this study was taken to determine the clinical profile, immunological and virological status of the HIV seropositive patients.

Review of Literature

<u>EPIDEMIOLOGY</u>

HIV infection/AIDS is a global pandemic with cases reported from virtually every country. At the end of 2007, 33.2 million individuals were living with HIV infection (range: 30.6–36.1 million) according to the Joint United Nations Programme on HIV/AIDS (UNAIDS).[1] More than 95% of people living with HIV/AIDS reside in low and middle income countries. 50% are females and 2.5 million are children less than 15 years.

In Asia, an estimated 4.9 million people were living with HIV infection at the end of 2007.[1] National HIV prevalence is highest in South East Asia with wide variation in trends between different countries. The epidemic in Asia lagged temporally behind that in Africa. However, the population of many Asian nations are so large (especially India and China) that even low national HIV prevalence rates result in large numbers of people living with HIV infection.

The HIV epidemic in India is concentrated in nature. The HIV prevalence among the High Risk Groups i.e. Female Sex Workers, Injecting Drug Users, Men who have Sex with Men and Transgenders is higher than the general population. In 2009 it was estimated that approximately 2.39 million people were infected with HIV in India, of which 39% were females and 4.4% were

children. The four high prevalence states of South India(Tamil Nadu, Kerala, Karnataka,Andhra Pradesh) account for 55% of these cases. Unprotected sex (87.4% heterosexual and 1.3% homosexual) is the major route of HIV transmission, followed by transmission from Parent to Child which is 5.4% and the use of infected blood and blood products which is 1.0%. While Injecting Drug Use is the predominant route of transmission in Northeastern states, it accounts for 1.7% of HIV infections nationally. [2]

HIV STRUCTURE AND GENOME

HIV is different in structure from other retroviruses. It is around 120 nm in diameter and spherical.HIV-1 is composed of two copies of single-stranded RNA enclosed by a conical capsid comprising the viral protein p24, typical of lentiviruses . The RNA component is 9749 nucleotides long.[3] The single-stranded RNA is tightly bound to the nucleocapsid proteins, p6, p7 and enzymes that are indispensable for the development of the virion, such as reverse transcriptase and integrase. The nucleocapsid (p7 and p6) associates with the genomic RNA (one molecule per hexamer) and protects the RNA from digestion by nucleases. A matrix composed of an association of the viral protein p17 surrounds the capsid, ensuring the integrity of the virion particle. Also enclosed within the virion particle are Vif, Vpr, Nef, p7 and viral Protease . The

envelope is formed when the capsid buds from the host cell, taking some of the host-cell membrane with it. The envelope includes the glycoproteins gp120 and gp41.

HIV has several major genes coding for structural proteins that are found in all retroviruses and several nonstructural ("accessory") genes that are unique to HIV. The *gag* gene provides the basic physical infrastructure of the virus, *pol* gene provides the basic mechanism by which retroviruses reproduce, while the others help HIV to enter the host cell and enhance its reproduction. Though they may be altered by mutation, all of these genes except *tev* exist in all known variants of HIV.

- *gag* (group-specific antigen): codes for the Gag polyprotein, which is processed during maturation to MA (matrix protein p17); CA (capsid protein p24); SP1 (spacer peptide 1 p2); NC (nucleocapsid protein p7); SP2 (spacer peptide 2 p1) and p6.

- *pol*: codes for viral enzymes reverse transcriptase, integrase, and HIV protease.

- *env* (for "envelope"): codes for gp160 - the precursor to gp120 and gp41 which are proteins embedded in the viral envelope. This enables the virus to attach to and fuse with target cells.

- Transactivators: *tat*, *rev*, *vpr*

- Other regulators: *vif*, *nef*, *vpu*

- *tev*: This gene is only present in a few HIV-1 isolates. It is a fusion of parts of the *tat*, *env*, and *rev* genes, and codes for a protein with some of the properties of tat, but little or none of the properties of rev.[4]

PATHOPHYSIOLOGY AND PATHOGENESIS

The hallmark of HIV disease is a profound immunodeficiency resulting primarily from a progressive quantitative and qualitative deficiency of the subset of T lymphocytes referred to as *helper T cells*. This subset of T cells is defined phenotypically by the presence of the CD4 molecule on its surface which serves as the primary cellular receptor for HIV. A co-receptor must also be present together with CD4 for efficient fusion and entry of HIV-1 into its target cells. HIV uses two major co-receptors for fusion and entry; these co-receptors are also the primary receptors for certain chemoattractive cytokines termed *chemokines* and belong to the seven-transmembrane-domain G protein–coupled family of receptors. CCR5 and CXCR4 are the major co-receptors used by HIV. A number of mechanisms responsible for cellular depletion and/or immune dysfunction of CD4+ T cells have been demonstrated in vitro; these include direct infection and destruction of these cells by HIV and immune clearance of infected cells, as well as indirect effects such as immune exhaustion due to aberrant cellular activation and activation-induced cell

death.[1]There are various stages in the lifecycle of HIV. This includes the following.

Binding and fusion:

HIV uses the CD4 molecule on the surface of the T lymphocyte as a primary receptor. Viral gp120 binds the CD4 molecule on the surface of the T lymphocyte leading to conformational change exposing the binding site for co-receptors (chemokine receptors) present on the surface of the T lymphocytes. The viral envelope fuses with the host cell membrane leading to the release of viral RNA copies in the protoplasm of the host cell.[5]

Reverse transcription:

Viral reverse transcriptase transcribes the single stranded HIV RNA to double stranded DNA that moves to the nucleus.[6]

Integration:

The enzyme integrase integrates the HIV DNA to host DNA. Integrated HIV DNA is called as provirus. The provirus may remain inactive for several years producing few or no copies of HIV. [7]

When host cell is activated, the provirus integrated in the host genome is also transcribed by host's RNA polymerase to create copies of HIV genomic material and also m RNA which produces the HIV proteins.

Assembly:

HIV protease cuts long chains of HIV protein into smaller individual proteins. HIV proteins with the genetic material form the new virus particles.[8]

Budding:

The newly assembled virus buds from the host cell. During budding , HIV envelope acquires host membrane protein and lipid bilayer.

NATURAL HISTORY OF HIV INFECTION

Acute stage of HIV infection (window period):

From the time of infection it may take 3-6 weeks for anti HIV antibodies to develop. This is called as the window period. There is a surge of plasma viremia with plasma viral load reaching peak in 2-3 weeks and loss of helper T cells in this period. The host generates immune response that helps in controlling the virus replication leading to sharp decline in plasma viremia. The patient presents with fever, arthralgia, myalgia, headache, rashes. This is self limiting. About 9-12 months post infection a steady state plasma viremia is reached (viral set point). This set point is prognostic.

Clinically latent phase of HIV infection:

The plasma viral load remains stable for several years post plasma viral load set point. Though viral load does not increase, there is constant multiplication of virus leading to destruction of CD4 lymphocytes. Based on the speed of disease progression HIV -1 infected individuals can be categorized as typical progressors, rapid progressors and long time non progressors. Typical progressors show a latent period of 5 -8 years before appearance of clinical AIDS. About 10% of the HIV infected rapidly progress to AIDS within 2-3 years of infection called as rapid progressors. 5% do not show clinical

symptoms even after 10 years of infection in the absence of ART called as long time non progressors.

AIDS stage:

In this stage, there is a rapid decline in CD4 count (<200cells) and patient acquires opportunistic infections and malignancies.[9]

LONG TERM NON PROGRESSORS:

Individuals infected with HIV for a long period (10 years or more), whose CD4 T cell counts are normal and have remained stable over years and who have not received antiretroviral therapy are regarded as long term non progressors. These patients have a low viral burden, normal immune function and normal lymphoid architecture. Other factors associated with this state include a defective virus that is of low virulence, defective nef gene and defective chemokine receptors.[10]

VIRAL DYNAMICS

The half-life of a circulating virion is approximately 30 to 60 min and that of productively infected cells is 1 day. Given the relatively steady level of plasma viremia and of infected cells, it appears that extremely large amounts of virus (10^{10} to 10^{11} virions) are produced and cleared from the circulation each day. In

addition, data suggest that the minimum duration of the HIV- 1 replication cycle in vivo is 2 days. Other studies have demonstrated that the decrease in plasma viremia that results from antiretroviral therapy correlates closely with a decrease in virus replication in lymph nodes, further confirming that lymphoid tissue is the main site of HIV replication and the main source of plasma viremia. The level of steady-state viremia, called the viral *set point*, at 1year has important prognostic implications for the progression of HIV disease. It has been demonstrated that HIV-infected individuals who have a low set point at 6 months to 1 year progress to AIDS much more slowly than individuals whose set point is very high at that time. Levels of viremia generally increase as the disease progresses. Measurement of the level of viremia is critical in guiding therapeutic decisions in HIV-infected individuals.[11]

CELLULAR TARGETS OF HIV

Although the CD4+ T lymphocytes and CD4+ cells of monocyte lineage are the principal targets of HIV, virtually any cell that expresses the CD4 molecule together with co-receptor molecules can potentially be infected with HIV. Circulating dendritic cells have been reported to express low levels of CD4 and depending on their stage of maturation, these cells can be infected with HIV. Epidermal Langerhans cells express CD4 and have been infected by HIV in vivo. HIV has been reported also to infect a wide range of cells and cell lines

that express low levels of CD4, no detectable CD4, or only CD4 mRNA. Among these are Functional dendritic cells, megakaryocytes, eosinophils, astrocytes, oligodendrocytes, microglial cells, CD8+ T cells, B cells, and NK cells as well as a variety of organ-specific cells.[12]

MECHANISMS OF CD4 T LYMPHOCYTE DESTRUCTION

- **Direct or single cell killing** : Infected CD4 cells may be killed directly when large number of virus is produced and bud off from the cell surface, disrupting the cell membrane, or when viral protein and nucleic acids collect inside the cell, interfering with cellular machinery.[13]

- **Syncytia formation** : Infected cells may fuse with nearby uninfected cells forming balloon like giant cells called syncytia.[14]

- **Apoptosis** : Infected CD4 cells may be killed when cellular regulation is altered by HIV proteins, probably leading to their suicide process known as apoptosis.[15]

- **Innocent Bystanders**: HIV particles may bind to the cell surface, making them appear as infected cells and marking them for destruction by killer T cells.

- **Anergy**: A negative signal is delivered to CD4 T cells after their component CD4 molecules react with gp120 or gp120-antigp120 complexes.[16]

- **Super antigens**: Viral protein nef is a super antigen, which activates the CD4 T cells for viral replication.[17]

- **Damage to precursor cells**: Studies suggest that HIV destroys precursor cells that mature to have special immune functions, as well as parts of the bone marrow and thymus needed for the development of such cells.

ROUTES OF HIV TRANSMISSION

Blood transfusion: 90-95%

Perinatal transmission: 20-40%

Sexual intercourse: 0.1- 10%

Vaginal intercourse: 0.05- 0.1%

Anal intercourse: 0.065 -0.5%

Oral sex: 0.005-0.01%

Injection drug abuse: 0.67%

Needle stick exposure : 0.3%

WHO STAGING

WHO clinical staging of HIV /AIDS of adults and adolescents (2006)

Clinical stage 1

- Asymptomatic

- Persistent generalized lymphadenopathy

Clinical stage 2

- Unexplained moderate weight loss (<10% of presumed or measured body weight)

- Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)

- Herpes zoster

- Angular cheilitis

- Recurrent oral ulceration

- Papular pruritic eruptions

- Seborrhoeic dermatitis

- Fungal nail infections

Clinical stage 3

- Unexplained severe weight loss (>10% of presumed or measured body weight)

- Unexplained chronic diarrhoea for longer than one month

- Unexplained persistent fever (above 37.5degree Celsius ,intermittent or constant for longer than one month)

- Persistent oral candidiasis

- Oral hairy leukoplakia

- Pulmonary tuberculosis

- Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)

- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

- Unexplained anaemia (<8 g/dl), neutropenia (<0.5 X 10^9/litre) and /or chronic thrombocytopenia(<50 X 10^9/litre)

Clinical stage 4

- HIV wasting syndrome

- Pneumocystis pneumonia

- Recurrent severe bacterial pneumonia

- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)

- Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

- Extrapulmonary tuberculosis

- Kaposi's sarcoma

- Cytomegalovirus infection (retinitis or infection of other organs)

- Central nervous system toxoplasmosis

- HIV encephalopathy

- Extrapulmonary cryptococcosis including meningitis

- Disseminated non-tuberculous mycobacterial infection

- Progressive multifocal leukoencephalopathy

- Chronic cryptosporidiosis

- Chronic isosporiasis

- Disseminated mycosis (extrapulmonary histoplasmosis, coccidiodomycosis)

- Recurrent septicaemia (including non-typhoidal salmonella)

- Lymphoma (cerebral or B cell non-Hodgkin)

- Invasive cervical carcinoma

- Atypical disseminated leishmaniasis

- Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

1. Assessment of body weight in pregnant woman needs to consider expected weight gain of pregnancy.

2. Unexplained refers to where the condition is not explained by other conditions.

3. Some additional specific conditions can also be included in regional classifications (e.g. reactivation of American trypanosomiasis

meningoencephalitis and/or myocarditis in Americas region, Penicilliosis in Asia).[18]

DERMATOLOGICAL MANIFESTATIONS OF HIV INFECTION

The cutaneous manifestations of HIV infection according to the CD4 count is as follows:

CD4 count	Dermatological manifestations
500 -1000	HIV exanthems in seroconversion illness, Tinea corporis, Psoriasis, Seborrheic dermatitis, Bullous impetigo.
200-500	Bacterial folliculitis, Pityriasis versicolor,Warts, Molluscum contagiosum, Herpes zoster.
< 200	Herpes simplex, Acquired icthyosis, Papular follicular eruptions, Oral candidiasis, Oral hairy leukoplakia, Kaposi's sarcoma, Norwegian scabies, Cryptococcosis, Penicilliosis, Tuberculosis.[19,20]

MARKERS OF DISEASE PROGRESSION

Viral load:

Plasma HIV RNA load is considered the most representative and sensitive laboratory test for monitoring progression of HIV infection and response to antiretroviral therapy.[21,22,23] Active replication occurs in all the stages. Viral load ranges between 10^2 and 10^7 copies /ml in untreated cases and is lower in those on treatment. Techniques available are RNA PCR, b DNA assay (branched chain DNA assay), NASBA (Nucleic acid sequence based amplification). RNA PCR detects 40 copies/ milliliter and is positive in > 98% of patients. b DNA assay detects 500 copies/ milliliter and is positive in >90% of the patients. While p24 antigen reflects changes in viral burden over an extended period, viral load measurement reflects the changes in HIV RNA levels over a few hours. Viral load levels are about ten fold lower in long time non progressors than in patients with progressive disease. Persistently detectable viremia and high baseline levels indicate poor prognosis. Risk of progression is low at copies less than 10,000 /ml. High viral load correlates with low baseline CD4 count, faster decline in CD4 count and faster disease progression.[24,25,26]

Interim recommendations for viral load assays:

Plasma HIV RNA levels that suggests initiation of treatment:

More than 5000-10,000 copies/ml and a CD4 count or clinical status suggestive of progressive disease.

Target level of HIV RNA level after initiation of treatment:

Undetectable : <5000 copies /ml is an acceptable target.

Minimal decrease in HIV RNA indicative of antiviral activity:

>0.5 log decrease.

Change in HIV RNA that suggests drug treatment failure:

Return to (or within 0.3 to 0.5 log of) pretreatment value, or a significant rise (0.7- 1.0 log) from the treatment nadir.

Suggested frequency of HIV RNA measurements:

At baseline, 2 measurements 2-4 weeks apart. Then every 3-4 months or in conjunction with CD4 count. 4-8 weeks after initiating or changing therapy.[27]

Serum p24 antigen:

HIV antigen detection tests use EIA technology for detection of HIV antigen in serum, plasma, urine. This can also detect p24 antigen in blood of HIV infected patients ,where it exists as free antigen or complexed to anti p24 antibody.[28,29,30,31] The sensitivity increases when the samples are treated with a weak acid to dissociate antigen antibody complexes prior to assay. Serum immune complex dissociated antigenemia was also associated with AIDS development. This is a less sensitive marker than plasma HIV RNA levels. During first few weeks of infection there is a brisk rise in p24 antigen level. After development of anti p24 antibody these antigen levels decline. Later in the course of the disease, when circulating levels of free virus are high, p24 antigen levels also increase.[32] Sensitivity of the test is 4% in asymptomatic , 56% in AIDS related complications, 70% in AIDS.[33] It has been shown that for a group of asymptomatic HIV infected patients with similar CD4 counts, those with a detectable level of p24 antigen are 3 times more likely on an average to show progression to AIDS than those in whom p 24 antigen levels cannot be detected. In patients on ART, there is a decline in circulating levels of p24 anigen. But it has a limited role in diagnosis and is not considered a useful prognostic marker.[21]

Serum p24 antibody:

Antibody to HIV p24 antigen begin to appear within several weeks of acute HIV infection. The level of p24 antibody declines because of formation of circulating immune complexes with p24 antigen. Thus a drop in titre of p24 antibody predicts an increase in viral load and increased risk of progression to AIDS.[34]

Assays for qualitative viral change:

Syncytium inducing strains:

Some HIV strains form syncytia or giant cells in certain continous cell culture lines. Syncytium inducing strains are more likely than non syncytium inducing strains to be seen at the end stage of infection, associated with increased viral load, a blunted CD4 response to ART, more rapid decline in CD4 count,drug resistance and progressive clinical deterioration. This assay is expensive and not routinely used.[35,36]

CD 4 cell count:

CD4 is one of the several glycoproteins termed as "cluster of differentiation antigens" expressed on the surface of lymphocytes . CD4 count can be measured by flow cytometry, microsphere assay and enzyme immunoassay. CD4 serves as a receptor for HIV and cells expressing this protein usually

decline in number with progressive HIV infection. Its decline is the hallmark of HIV infection. CD4 cell count is extremely important in staging of HIV infection and a revised classification of the centre for disease control divide HIV positive patients into 3 CD4 count categories. > 500/ microlitre, 200-499/ microlitre,< 200/microlitre. A low CD4 count (<10%), number < 100/microlitre, and a CD4 /CD8 ratio (<0.2) are highly predictive for death from AIDS related complications.[37,38]

Percentage of CD4 cells:

The total number of CD4 cells in any one sample are influenced by specimen handling , age of the patients , time of sampling(low count in the morning), use of pharmacological agents and presence of infection. So more value is given to percentage of CD4 cells as a marker of immune function and progression to AIDS. Normally CD4 count of 200/microlitre is equivalent to 20% of lymphocyte count. The normal range of CD4 count is 500- 1300/ microlitre and percentage is 38-65%. When CD4 count varies unexpectedly or clinical stage does not correlate with CD4 count, then CD 4 percentage and its variation over time should be monitored.[39]

CD8 cell count:

It is not as useful as CD4 count as it remains elevated for many years after infection. However in advanced immunodeficiency (CD4 < 200), a marked decline in CD8 levels indicate poor prognosis.[22,34]

Multitest delayed type hypersensitivity skin test:

Semiquantitative measurement of immune function. Lack of response (anergy) is associated with a poor prognosis. Testing involves placement of a panel of 7 purified recall antigens on the volar aspect of the forearm with the diameter of induration of each reaction measured 48 hours later. Reactions > 2mm diameter is considered as positive. Failure to respond to any antigen is considered as anergy and indicates poor cell mediated immunity. The use of gp160 in a delayed type hypersensitivity test panel is being investigated.[22,34]

Soluble markers:

Neopterin:

Neopterin is a low molecular weight protein derived from an intermediate product of denovo biosynthesis of tetrahydrobiopterin from guanosine triphosphate. Its produced by activation of monocytes and B cells. It is an early marker of HIV infection.[21,23,34,40] Its levels further rise on progression from pre AIDS to clinical AIDS. It is a non specific marker as it is also positive in viral

infections, advanced malignancies, collagen vascular disorders, atypical phenylketonuria, patients on immunostimulants. Neopterin assay can be done by ELISA or RIA .Serum or urine levels of neopterin are higher in patients with advanced HIV infection and in asymptomatic patients higher levels are associated with increased progression to AIDS that is independent of CD4 count.[41,42,43] In the San Francisco cohort study, patients with neopterin levels < 12nmol/l, 12-17nmol/l, >17nmol/l had 3 years rate of progression to AIDS of 10%, 20%, 45% respectively.[39]

Beta 2 microglobulin:

It is a 11kDa protein that is expressed on the surface of most nucleated cells. It forms a heterodimer with class 1 MHC molecules present on the surface of most nucleated cells. It also exhibits aminoacid homology with the constant region of immunoglobulin. Free beta 2 microglobulin can be measured in the serum and urine and correlates with the progression of HIV infection. Levels of beta 2 microglobulin are increased in conditions characterized by lymphocyte activation or destruction such as lymphoproliferative syndromes, autoimmune diseases, viral infections, renal diseases, hemophiliacs, drug abusers. It can be measured by RIA or ELISA.[44] Increased beta 2 microglobulin levels are associated with decreased CD4 cell counts. In the Sanfrancisco cohort study, beta 2 microglobulin levels <3 mg/l, 3-5mg/l, > 5mg/l had 3 years of progression to AIDS of 12%, 33%, and 69% respectively.[45,46,47]

Other markers:

Serum IgA levels[48]

Serum cytokine levels (TNF α and IFN)[23,34]

Soluble IL- 2 receptor levels.[49]

<u>DIAGNOSIS</u>

HIV infection can be detected in the laboratory either by detection of antibodies to HIV or by detection of virus, its antigen and its DNA or RNA.

Antibody detection:

Screening tests:

ELISA (Enzyme linked immunosorbent assay)

It is the most commonly performed test.

The types of ELISA based on the principle are indirect ELISA, competitive ELISA, sandwich ELISA, Immunocapture ELISA.

ELISA can also be classified based on the antigen utilized into first generation, second generation ,third generation, fourth generation.

First generation – infected cell lysates is used as antigen.

Second generation – glycopeptides is used as antigen.

Third generation – synthetic peptides are used as antigen.

Fourth generation – antigen and antibody are detected simultaneously.

Rapid tests:

This includes the following tests:

1. Dot blot assays (Immunoconcentration based)

2. Particle agglutination (Gelatin, RBC, latex, microbeads)

3. Dipstick and COMB tests (ELISA technology based)

4. Immunochromatography based tests (lateral flow of reagents)

Supplemental tests:

These are the following.

1. Second and third generation ELISA / rapid tests.

2. Western blot.

Detection of p24 antigen:

This is done in the following conditions:

1. To detect infection in the newborns

2. To resolve equivocal Western blot results.

3. To detect infection during early window period.

4. To diagnose CNS Infection.

5. Late stages of the disease.

6. For research purposes.

7. To monitor response to antiretroviral therapy.

Viral culture:

It is another method for identifying HIV infection. But it is expensive and labour intensive. It is mainly used for research purposes.

STRATEGIES OF HIV TESTING

The various strategies designed involve the use of various categories of tests in various permutations and combinations.

1. Screening tests – ELISA/ rapid tests (E/R).

2. Supplemental tests E/R and Western blot.

Western blot is used where discordant serological results are obtained. If Western blot is not available, then client should be retested again after 2-4 weeks.

Strategy 1:

Blood/serum/plasma is subjected once to E/R for HIV. If negative, the sample is considered free of HIV and if positive, the sample is taken as HIV infected for all practical purposes. This strategy is used for ensuring donation safety (blood, organs, tissues, sperms).

Strategy 2 A:

A serum sample is considered negative for HIV if the first ELISA or rapid test is negative , but if reactive the same sample is subjected to a second ELISA or rapid test which utilises a different system from the first one , that is either the principle of the test /and/or the antigen used is different. It is reported reactive only if the second ELISA / rapid test confirms the positive report of the first test. In case the second E/R is nonreactive, then the test result is taken as negative for surveillance purposes. This strategy is used for sentinel surveillance.

Strategy 2 B:

This strategy is used for detection of HIV infection in symptomatic individuals with symptoms of AIDS clinically. A sample reactive with the first assay and nonreactive with the second assay is subjected to the third test E/R. If the third test E/R is reactive , the sample is reported as indeterminate and the patient is called for repeat testing after 2-4 weeks. If the third test is negative, it is

reported as negative. In this strategy if the first 2 consecutive tests are positive, a positive report can be given to the patient.

Strategy 3:

Testing is done as in strategy 2 B. However added confirmation of a 3rd E/R reactive test is required for a sample to be reported as HIV positive .If the sample gives reactive result with 2 E/R and nonreactive with the third assay, it is reported as indeterminate and the patient is called back for repeat testing after 2- 4 weeks . The test used as the screening test is of high sensitivity and the supplemental test is of high specificity. This strategy is used for diagnosis of HIV infection in asymptomatic individuals.[50]

LABORATORY DIAGNOSTIC METHODS

RETROCHECK:

 It is a third generation , rapid, qualitative, two side sandwich immunoassay for the simultaneous detection of antibodies specific to the HIV 1 and HIV 2 virus in serum, plasma or whole blood. The test employs recombinant proteins representing the highly immune dominant envelope and core regions of HIV 1 (gp41 and p24-0- subtype fusion polypeptide) and the envelope region of HIV 2 (gp36). The principle used is lateral flow immunochromatography, a 2 site double antigen sandwich immunoassay on a membrane. As the test specimen flows through the membrane of the test assembly, the coloured HIV specific

recombinant antigen- colloidal gold conjugate complexes with HIV antigen in the sample. This complex moves further on the membrane of the test region 'T' where it is immobilized by the HIV specific recombinant antigen coated on the membrane leading to the formation of a coloured band which confirms a positive test result. Absence of the coloured band in the test region 'T' indicates a negative test result. The unreacted conjugate and unbound complex if any, move further on the membrane and are subsequently immobilized by the antirabbit antibodies coated on the membrane at the control region 'C' forming a coloured band. This control band serves to validate the reagent and the test performance.[51]

SD BIOLINE HIV ½ 3.0:

SD BIOLINE HIV ½ 3.0 test is an immunochromatographic test for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to HIV -1 and HIV -2 simultaneously in human serum , plasma or whole blood.This test contains a membrane strip precoated with recombinant HIV -1 capture antigen(gp41,p24) on test band 1 region and recombinant HIV -2 capture antigen(gp36) on test band 2 region respectively. The recombinant HIV-1/2 antigen (gp41,p24, gp36) – colloidal gold conjugate and the specimen sample move along the membrane chromatographically to the test region (T)and form a visible line as the antigen – antibody- antigen gold particle complex forms with high degree of sensitivity and specificity.[52]

COMBAIDS:

COMBAIDS - RS advantage is a dot immunoassay for the detection of antibodies to HIV -1 and HIV -2 in the whole blood , human serum, plasma. This is an indirect solid phase enzyme immunoassay. The solid phase is a comb with 12 projections. Each tooth has 3 spots. Upper spot: goat antibody to human Ig. Middle spot : HIV-1 synthetic peptides. Lower spot : HIV-2 synthetic peptides. The developing plate has 6 rows (12 wells each) with each row containing a reagent solution ready for use at different steps in the assay. Ig solution present in the test samples as captured by the antihuman Ig antibody on the upper spot (internal control). Unbound components are washed away. IgG from the sample is captured on the teeth and reacts with antihuman IgG antibody labelled with alkaline phosphatase which reacts with chromogenic components and the results are seen as gray- blue spots on the surface of the teeth of the comb.

Interpretation of controls:

Appearance of upper spot: negative control.

Appearence of all 3 spots: positive control.

Upper spot does not appear: invalid result.

Interpretation of results:

Appearance of upper spot: nonreactive sample

Appearance of upper and middle spot: reactive for HIV-1.

Appearance of upper and lower spot: reactive for HIV-2 .

Appearance of all the 3 spots: reactive for HIV-1 and HIV-2.[53]

CD4 COUNT DETERMINATION BY FLOW CYTOMETRY:

Flow cytometry refers to a technology that simultaneously measures and analyses multiple physical and chemical characteristics of single cells or other biological particles, as they flow in a fluid stream past optical and / or electronic sensors. It provides information about their relative size , relative granularity or internal structure and fluorescence in several spectral regions emitted by fluorochrome labelled probes which bind specifically and stoichiometrically to cellular constituents such as protein and nucleic acid. Individual cells stained with fluorescent labels or absorption dyes are suspended in a physiological solution and introduced under a slight pressure through a flow chamber into the centre of a stream of cell free sheath fluid. The light scattered by the individual particle and the fluorescence emitted by the cells is used for analysis and sorting of the cells based on the fluorescence antibody directed against a specific surface antigen. This combination of scattered and fluorescent light is picked up by the detectors in the flow cytometer. These detectors then produce electronic signals that are proportional to the optical signals received.

The visible light undergoes deflection based on the size and the internal structure of the cells. Forward scatter correlates with cell volume. Side scatter depends on the shape of the nucleus, amount and type of cytoplasmic granules. The fluorescence emitted by the cells depends on the flourescence tagged specific monoclonal antibodies- antigen cell surface markers. The data collected on each cell is stored in the computer. This data is then processed and analysed to provide information about all cell populations within the sample.[54,55]

REAL TIME HIV-1 PCR ASSAY:

The RealTime HIV-1 assay amplifies a fragment of 172 nucleotides from a highly conserved region of the integrase gene. The assay incorporates an internal control (IC) that is unrelated to the HIV-1 target sequence and the hydroxypyruvate reductase gene from the pumpkin plant (*Cucurbita pepo*), which is introduced into each specimen at the beginning of the sample preparation. During each round of thermal cycling, amplification products dissociate to single strands at a high temperature, allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures. Amplification of both targets, HIV-1 and IC, takes place simultaneously in the same reaction. The hybridization of the HIV cDNA molecules and IC-specific probes, labeled with a different fluorescent dye, using

lower temperatures than primer annealing allows the best detection of different HIV variants. The amplification cycle at which a fluorescent signal is detected is proportional to the amount of HIV-RNA present in the original sample. The lower limit of detection is defined as the HIV-RNA concentration detected with a probability of 95% or greater; depending on the sample volume used for RNA isolation. A lower limit of detection of 40 HIV-RNA copies/ml has been established when RNA is isolated from 1 ml of plasma. [56]

Aim of the Study and Materials & Methods

AIM

To determine the clinical,virological and immunological status of HIV positive patients who attend Institute of Venereology and ART centre at Madras Medical College, Chennai.

MATERIALS AND METHODS

Study design: Cross sectional study

Study period: May 2010 - August 2011

Subject Selection: 50 treatment naive HIV positive patients diagnosed at Institute of Venereology and ART Centre at Madras Medical College ,Chennai were randomly selected for the study.

Inclusion Criteria:

- Patients who were positive for HIV infection.

- Patients who were at the various stages (WHO staging) of HIV disease.

Exclusion criteria

- HIV positive patients who were on ART.

Study:

Patients selected for study were subjected to detailed clinical history,examination and necessary routine laboratory investigations. Clinical staging was done according to the WHO clinical staging for AIDS.

At recruitment, blood samples were taken from all the patients in a plain vial for serum HIV testing and EDTA vacutainer for whole blood CD4 cell count and plasma viral load determination after obtaining informed consent.

Laboratory methods:

- Serodiagnosis of HIV infection will done by

    Test 1 –RETROCHECK

    Test 2- SD BIOLINE HIV ½ 3.0

    Test 3- COMBAIDS

    Strategy used: Strategy 3

- Absolute CD4 lymphocyte count determined by Flow cytometry

- Viral load done using Real-Time PCR :

    Real-Time PCR based HIV Assay was standardized and done by HELINI Biomolecules.

Observations

RESULTS

VIROLOGICAL, IMMUNOLOGICAL AND CLINICAL STATUS OF THE PATIENTS:

	WHO stage	Patient ID	CD 4 count/µl	HIV RNA copies/ml
1.	Stage I	P001	419	12388
2.		P003	327	23129
3.		P004	324	80252
4.		P006	705	23346
5.	19 patients	P007	368	67816
6.		P011	390	23002
7.		P013	921	41344
8.		P015	447	77231
9.		P016	519	3463
10.		P018	226	372033
11.		P020	368	458492
12.		P026	308	30034
13.		P028	459	18990
14.		P032	503	12542
15.		P036	223	142971
16.		P040	309	43671
17.		P046	466	12899
18.		P047	327	38862
19.		P048	390	331241
20.	Stage II	P005	302	3359
21.		P008	438	1973
22.		P009	488	2200
23.	10 patients	P017	505	212
24.		P022	356	3459
25.		P031	412	1183
26.		P038	342	1114
27.		P041	220	7114
28.		P045	492	3981
29.		P049	356	2345

	WHO stage	Patient ID	CD 4 count/µl	HIV RNA copies/ml
30.	Stage III	P002	55	8542
31.		P010	131	31203
32.	18 patients	P012	68	9243
33.		P014	447	2334
34.		P019	386	1276
35.		P021	211	29842
36.		P023	443	1370
37.		P025	364	16711
38.		P024	268	1263
39.		P027	339	3331
40.		P029	333	4457
41.		P033	301	11932
42.		P034	254	100
43.		P035	184	7112
44.		P042	65	29022
45.		P043	296	40021
46.		P044	493	1459
47.		P050	339	14921
48.	Stage IV	P030	38	197432
49.	3 patients	P037	92	49025
50.		P039	53	131315

DISTRIBUTION OF PATIENTS IN DIFFERENT STAGES

WHO Staging	No of patients	Percentage
I	19	38%
II	10	20%
III	18	36%
IV	3	6%

- Maximum number of patients were in WHO stage I (38%).

- 36% of the patients were in WHO stage III.

- 20% of the patients were in WHO stage II.

- Only 6% of the patients were in WHO stage IV.

DISTRIBUTION OF PATIENTS IN DIFFERENT STAGES
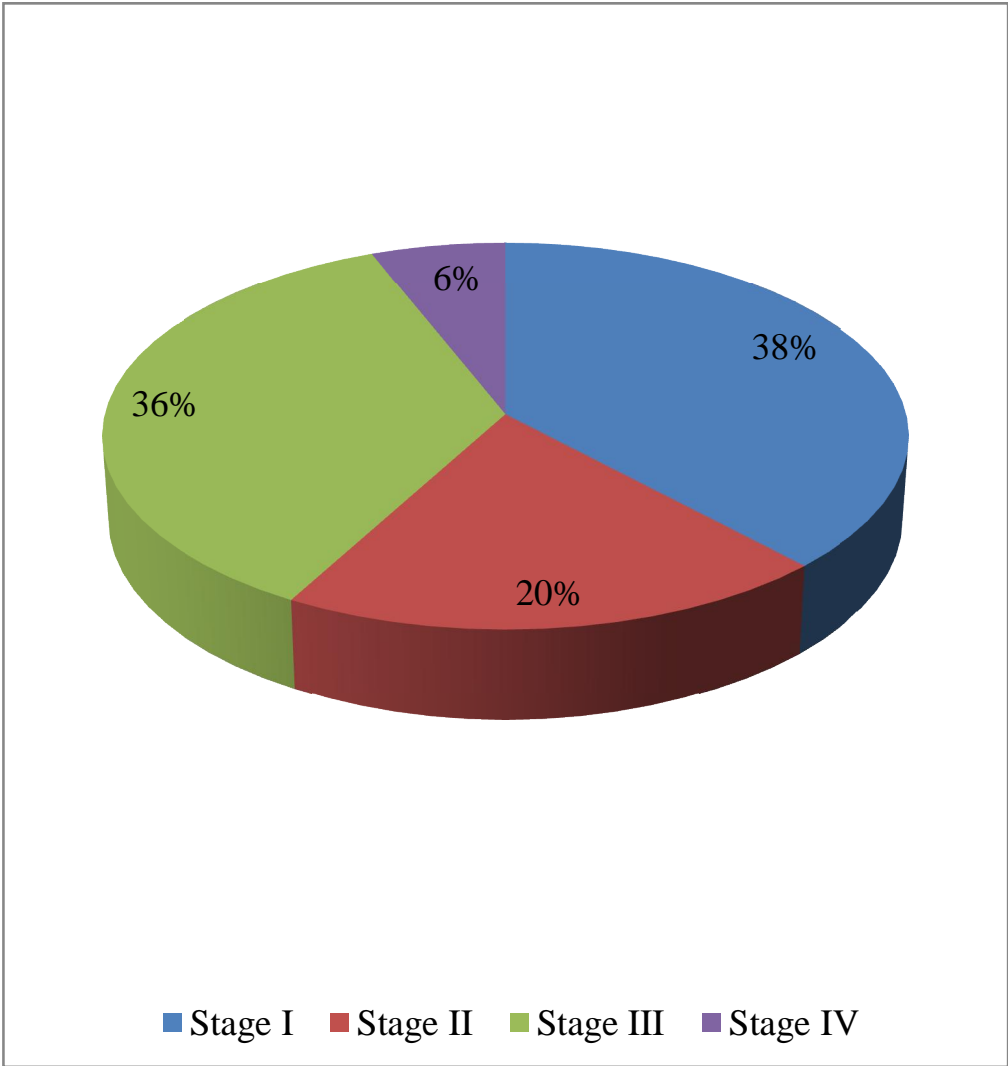
RANGES OF ABSOLUTE CD4 LYMPHOCYTES PER µl IN HIV INFECTED INDIVIDUALS

CD 4 count ranges in cells /µl	WHO staging- no of patients				Total no of patients	Percentage
	Stage I	Stage II	Stage III	Stage IV		
>500	4	1	0	0	5	10%
350-500	8	6	5	0	19	38%
200-349	7	3	8	0	18	36%
50-199	0	0	5	2	7	14%
<50	0	0	0	1	1	2%

Mean CD 4 count in cells /µl	421	391	276	61

MEAN CD4 COUNTS IN DIFFERENT STAGES

MEAN CD4 COUNT

421	stage I
391	Stage II
276	stage III
61	stage IV

Y-axis: MEAN CD4 COUNT (0, 50, 100, 150, 200, 250, 300, 350, 400, 450)

X-axis: STAGES
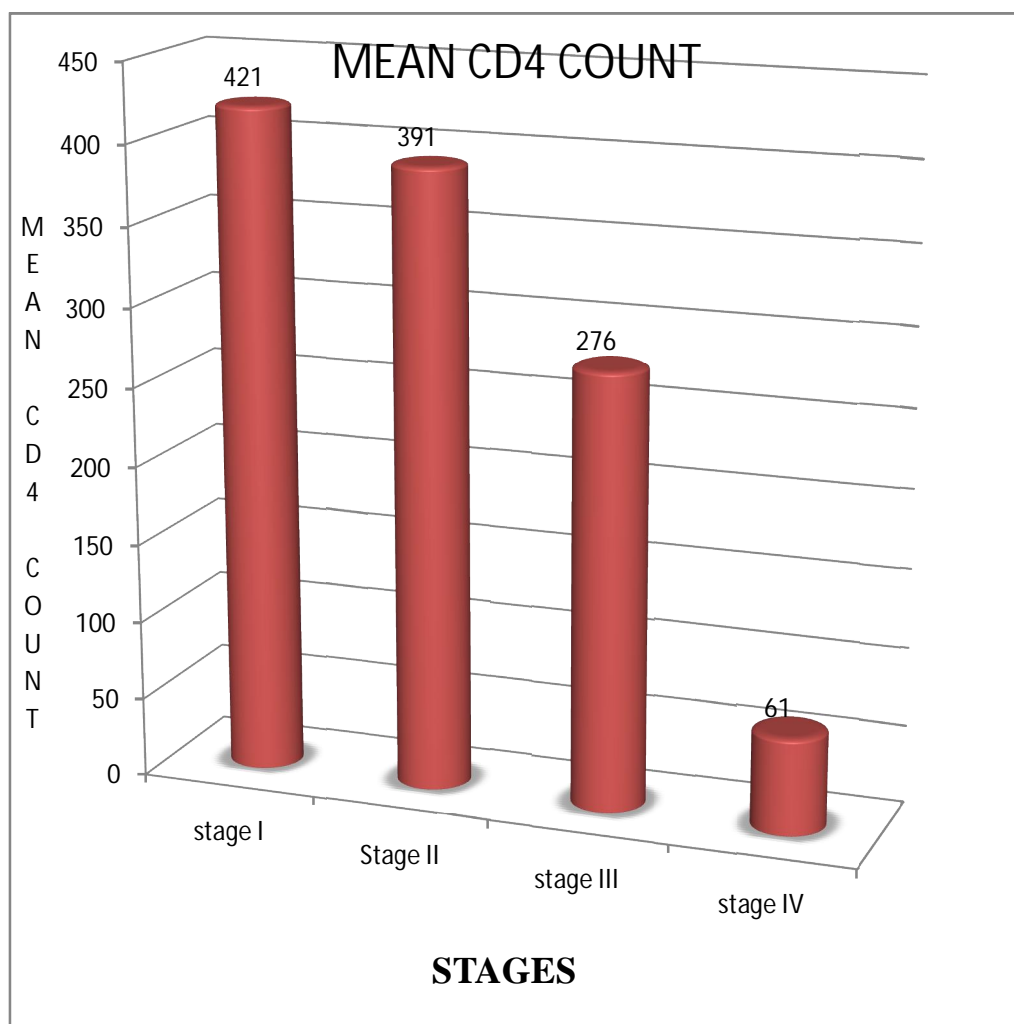
- 38% of the patients had CD4 count between 350-500 cells/ µl.

- The CD4 count range was between 200-349 cells/ µl in 36% of the patients.

- 14% of the patients had CD4 count between 50-199 cells/ µl.

- 10% of the patients had a CD4 count more than 500 cells/µl.

- Only 2% of the patients had CD4 count <50 cells/µl.

The mean CD4 counts were found to be 421, 391, 276, 61 cells/µl in the WHO stages I, II, III and IV respectively. The CD4 count ranged between 38-921 cells/µl. The mean CD4 count was found to be 341.4 cells/ µl. There is no statistically significant difference between the mean CD4 count between the stages I and II (p value = 0.6013). There is a significant difference between the mean CD4 count between stages I and III (p value = 0.0062). There is a significant difference between the CD4 counts in stages 1and 4(p value = 0.0014). A statistically significant difference (p value = 0.0244) is found between the CD4 counts of stages II and IV. There is a statistically significant difference in the CD4 counts of stages II and IV (p value <0.0001). On analyzing the mean CD4 counts of stages III and IV, a statistically significant difference is found (p value= 0.0139).

RANGE OF VIRAL LOAD COPIES PER ml IN HIV INFECTED PATIENTS

Viral load ranges	WHO staging- no of patients				Total no of patients	Percentage of patients
	Stage I	Stage II	Stage III	Stage IV		
$<1.5 \times 10^3$	0	3	5	0	8	16%
$1.5\text{-}7 \times 10^3$	1	6	3	0	10	20%
$7\text{-}20 \times 10^3$	4	1	6	0	11	22%
$20\text{-}55 \times 10^3$	8	0	4	1	13	26%
$>55 \times 10^3$	6	0	0	2	8	16%

Mean viral load	95458	2694	11897	125924

MEAN VIRAL LOAD IN DIFFERENT STAGES

Viral load

140000

120000

100000

80000

60000

40000

20000

0

MEAN VIRAL LOAD

95458

2694

11897

125924

■ Viral load

stage I

stage II

stage III

stage IV

STAGES
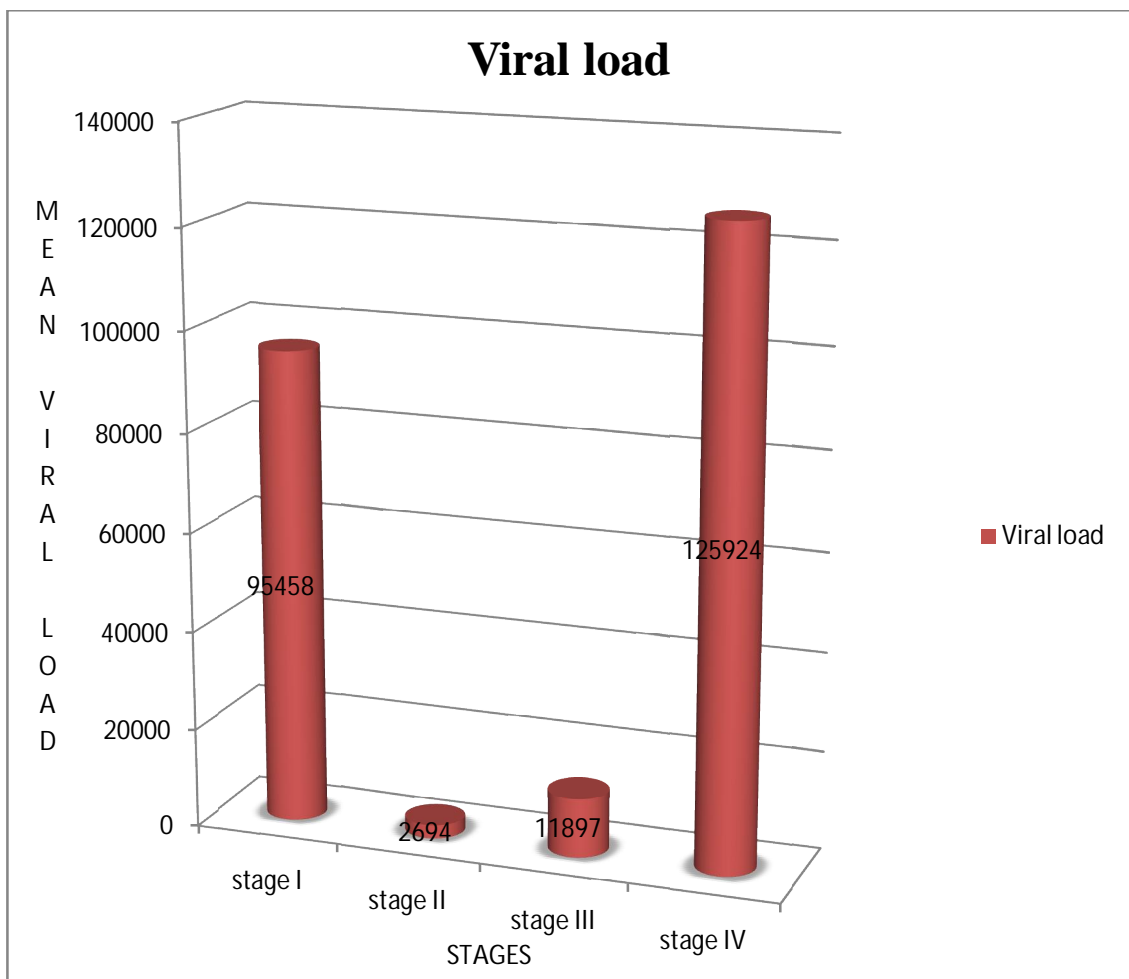
- 26% of the patients had viral load between 20-55$\times10^3$/ml.

- 22% of the patients had viral load ranging between 7-20 $\times10^3$/ml.

- The viral load range was between 1.5-7$\times10^3$/ml in 20% of the patients.

- 16% of the patients had a viral load $> 55 \times10^3$/ml.

- Viral load of less than 1.5$\times10^3$/ml was seen in 16% of the patients.

The viral load ranged between 1×10^2 to 4.58×10^6 copies/ml. The mean viral load was found to be 4.8×10^4 copies/ml. There is a statistically significant difference in the viral load between stage 1 and 2 (p value =0.0412). A significant difference is observed in the viral load between stage 1 and 3 (p value = 0.0135). There is no significant difference in the viral loads between stages 1 and 4 (p value = 0.7117). A significant difference in the viral load is observed between stages 2 and 3 (p value = 0.0302). Stages 3 and 4 showed a significant difference in viral load (p value <0.0001).

OPPORTUNISTIC INFECTIONS IN DIFFERENT CD4 COUNT CATEGORIES

OPPORTUNISTIC INFECTIONS	CD 4 count- no of patients					Total
	<50	50-200	200-350	350-500	>500	
Pulmonary Tuberculosis	0	4	3	2	0	9
Oral candidiasis	0	5	5	4	0	14
Infective diarrhoea	0	2	0	2	0	4
Recurrent URI	0	0	1	1	0	2
Herpes zoster	0	2	0	1	0	3
Oesophageal candidiasis	1	1	0	0	0	2
Extra pulmonary Tuberculosis	1	0	0	0	0	1

- The most common opportunistic infection was found to be oral candidiasis, seen in 28% of the patients.

- This was followed by pulmonary tuberculosis seen in 18% of the patients.

TUBERCULOSIS IN DIFFERENT CD4 CATEGORIES

CD4 count	No of patients with tuberculosis	Percentage
>500	0	0%
350-500	2	4%
200-350	3	6%
50-199	4	8%
<50	1	2%

- A total number of 10 patients were found to have tuberculosis.

- None of the tuberculosis patients had a CD4 count > 500 cells/µl.

- A maximum of 8 patients had CD4 count < 350 cells/ µl.

- Of the 10 patients, one of them had extrapulmonary tuberculosis (CNS tuberculosis). This patient had a CD4 count < 50 cells/µl.

CORRELATION BETWEEN VIRAL LOAD AND CD4 COUNT
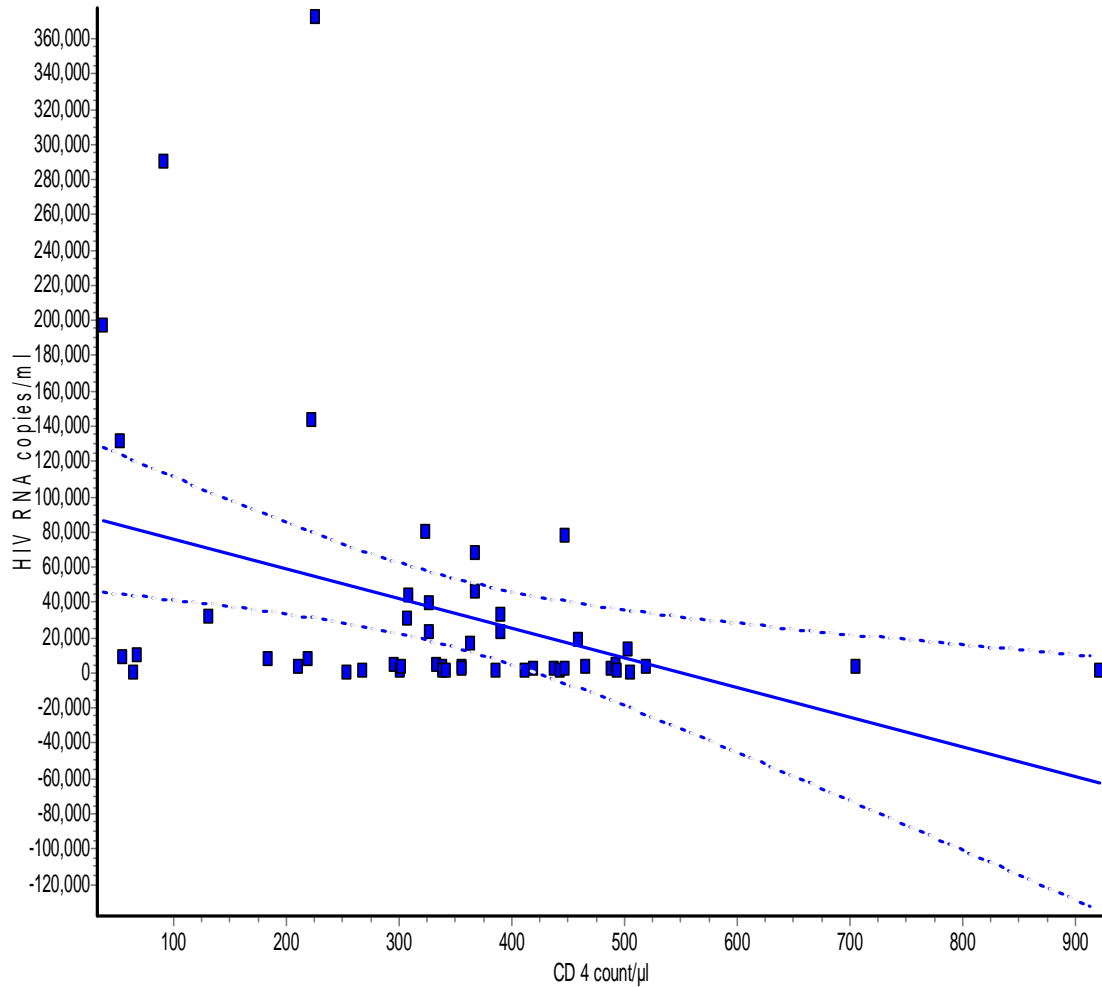
Correlation coefficient = -0.3800 (P= 0.0065)

There was a significant correlation between increasing HIV RNA levels and decreasing CD4 counts.

AGE DISTRIBUTION IN THE STUDY

Age group (in years)	No of patients	Percentage
Birth -12 years	0	0%
13-20 years	1	2%
20-50 years	43	86%
>50 years	6	12%

- 86% (43) of the patients belong to the 20-50 years age group.

- 12% (6) of the patients were above 50 years of age.

- 2% (1) of the patients were in the 13- 20 years age group

AGE DISTRIBUTION IN THE STUDY

2%

12%

86%

■0-12 years ■13-20 years ■20-50 years ■ > 50 years
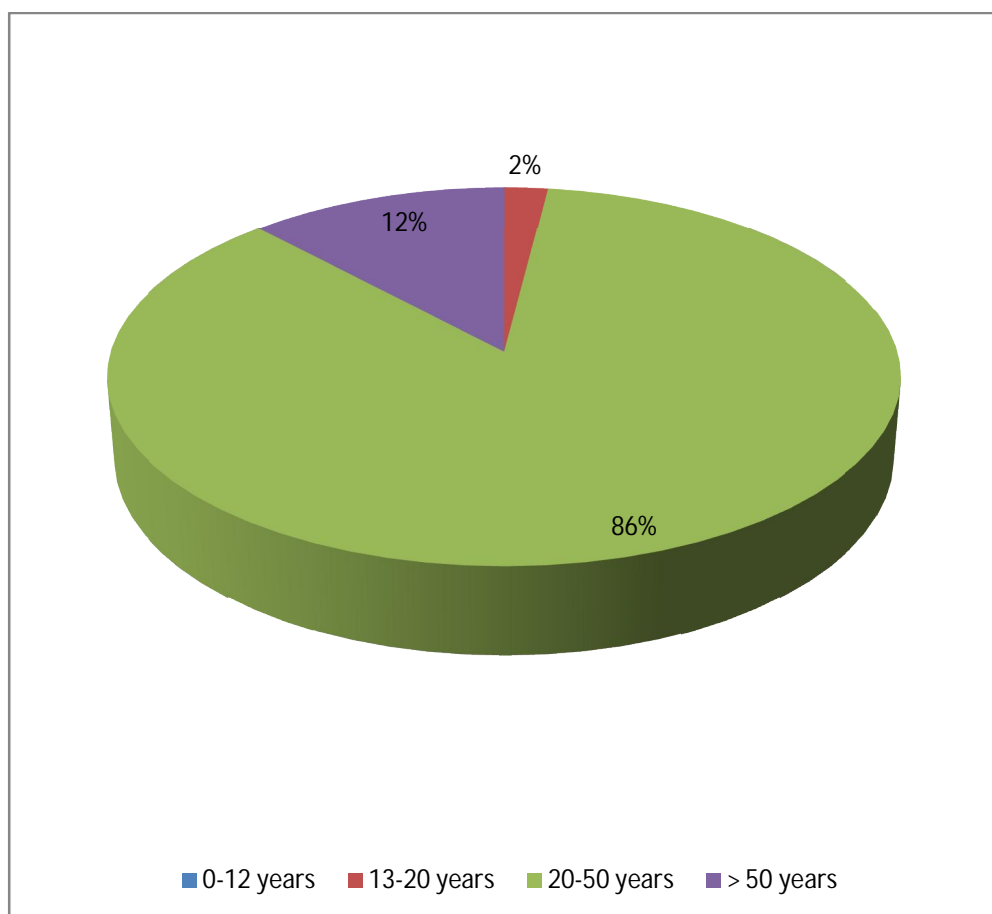
SEX DISTRIBUTION IN THE STUDY

Males –29   (58%)

Females –20   (40%)

Transgender -1 (2%)
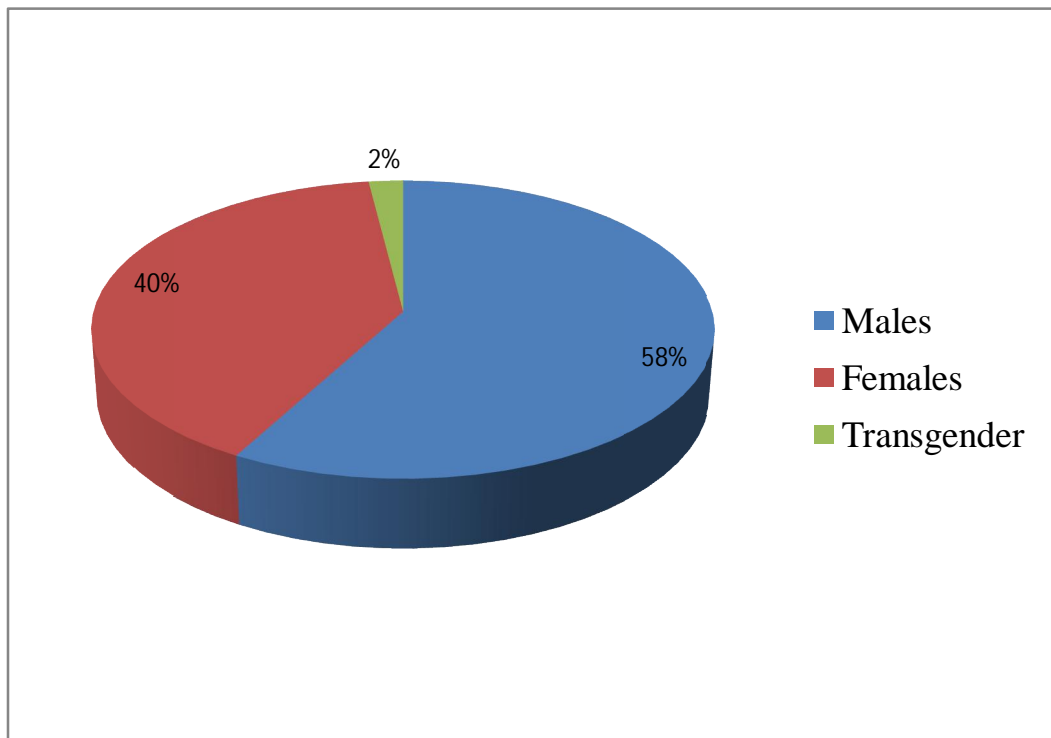
RISK FACTORS FOR HIV ACQUISITION

Risk factor	No of patients	Percentage
Heterosexual high risk behaviour	45	90%
Heterosexual with Injection drug abuse	2	4%
Homosexual	1	2%
Mother to child transmission	1	2%
Bisexual	1	2%

- 45(90%) of the patients showed heterosexual high risk behavior alone.

- 1 (2%) of the patients showed homosexual behavior.

- Heterosexual high risk behavior with Injection drug abuse was found in 2 (4%) of the patients.

- Mother to child transmission was seen in 1 (2%) patient.

- One (2%) of the patients showed Bisexual high risk behavior.

RISK FACTORS FOR HIV ACQUISITION

- Heterosexual high risk behavior
- Homosexual
- Mother to child transmission
- Heterosexual with injection drug abuse
- Bisexual
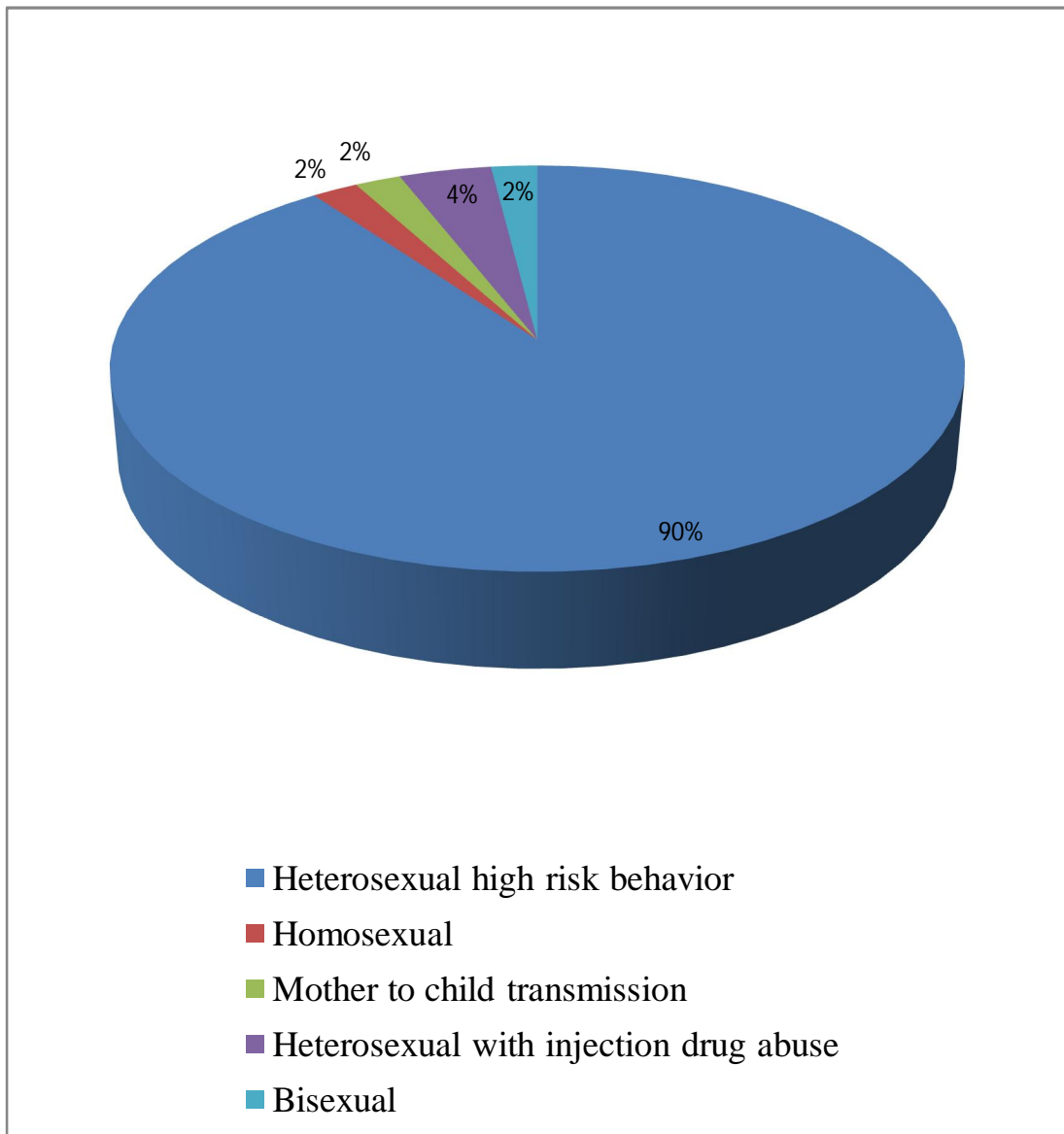
HBV STATUS

No of positives: 7(14%)

No of negatives: 43(86%)

43 of the patients were negative for HBV. 7 of them were positive for HBV.
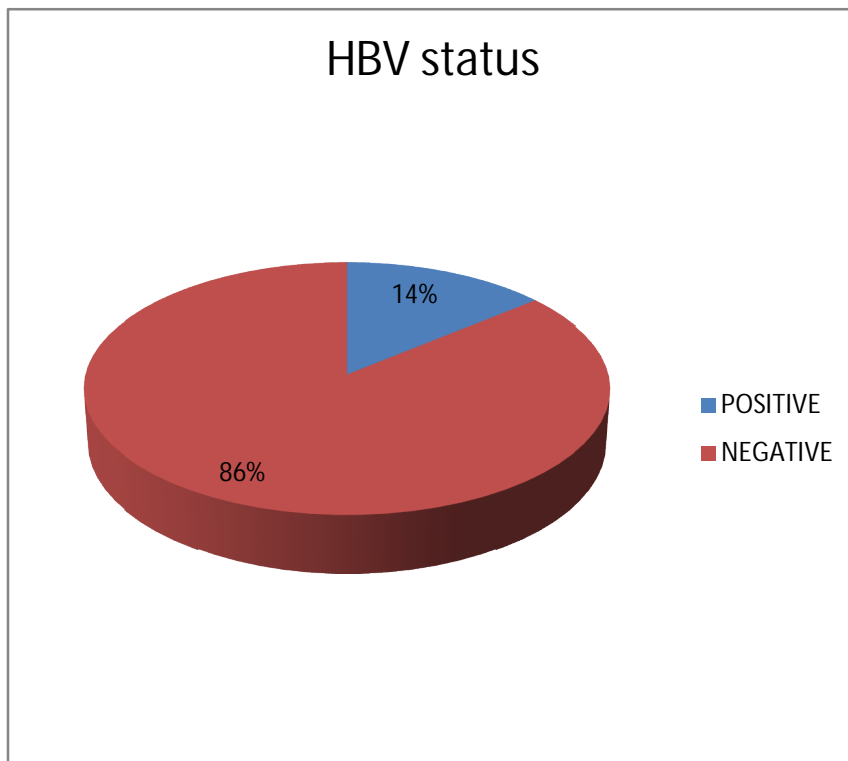
CLINICAL PRESENTATION

Clinical presentation (symptoms and signs)	No of patients	Percentage
Asymptomatic	17	34%
Weight loss	7	14%
Diarrhoea	4	8%
Fever	12	24%
Asthenia	2	4%
Cough	10	20%
Dermatoses	28	56%

- The most common clinical presentation was various dermatoses (56%).

- 34% of the patients were asymptomatic.

- Fever was seen in 24% and cough in 20% of the patients.

- 14% of the patients presented with weight loss.

- Diarrhoea was seen in 8% of the patients.

- 4% of the patients had asthenia as the presenting symptom.

CLINICAL PRESENTATION

Clinical presentation
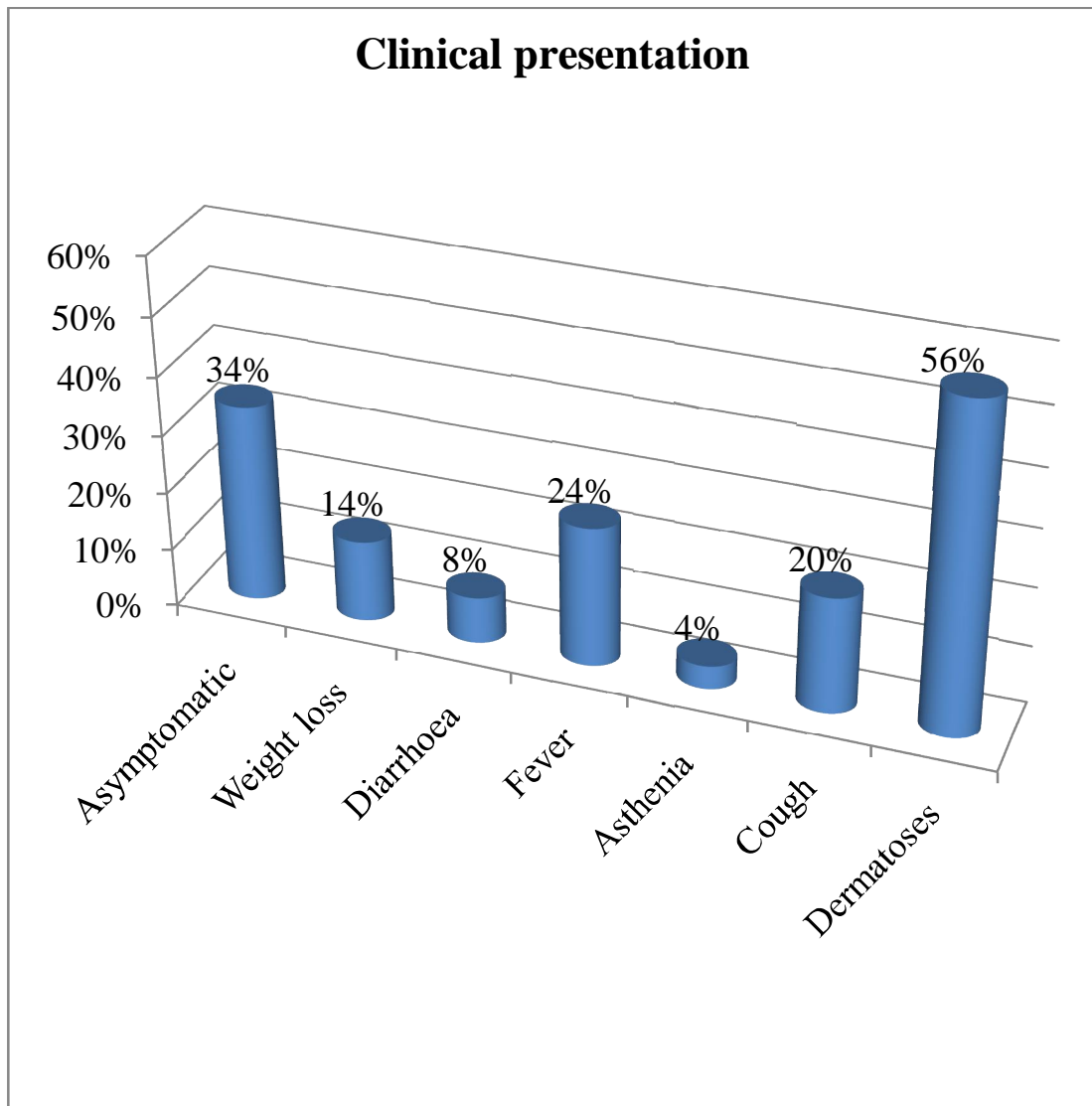
SEXUALLY TRANSMITTED INFECTIONS

Sexually transmitted infections	No of patients
Syphilis (VDRL Positivity)	4
Genital herpes	1
Perianal Wart	1
Vulvovaginal candidiasis	4
Trichomoniasis	2
Bacterial vaginosis	1

- In this study 4 patients had Syphilis (VDRL Reactive) out of which 3 were males and 1 was a female.

- Vulvovaginal candidiasis is the most common STI co-infection among females.

SEXUALLY TRANSMITTED INFECTIONS
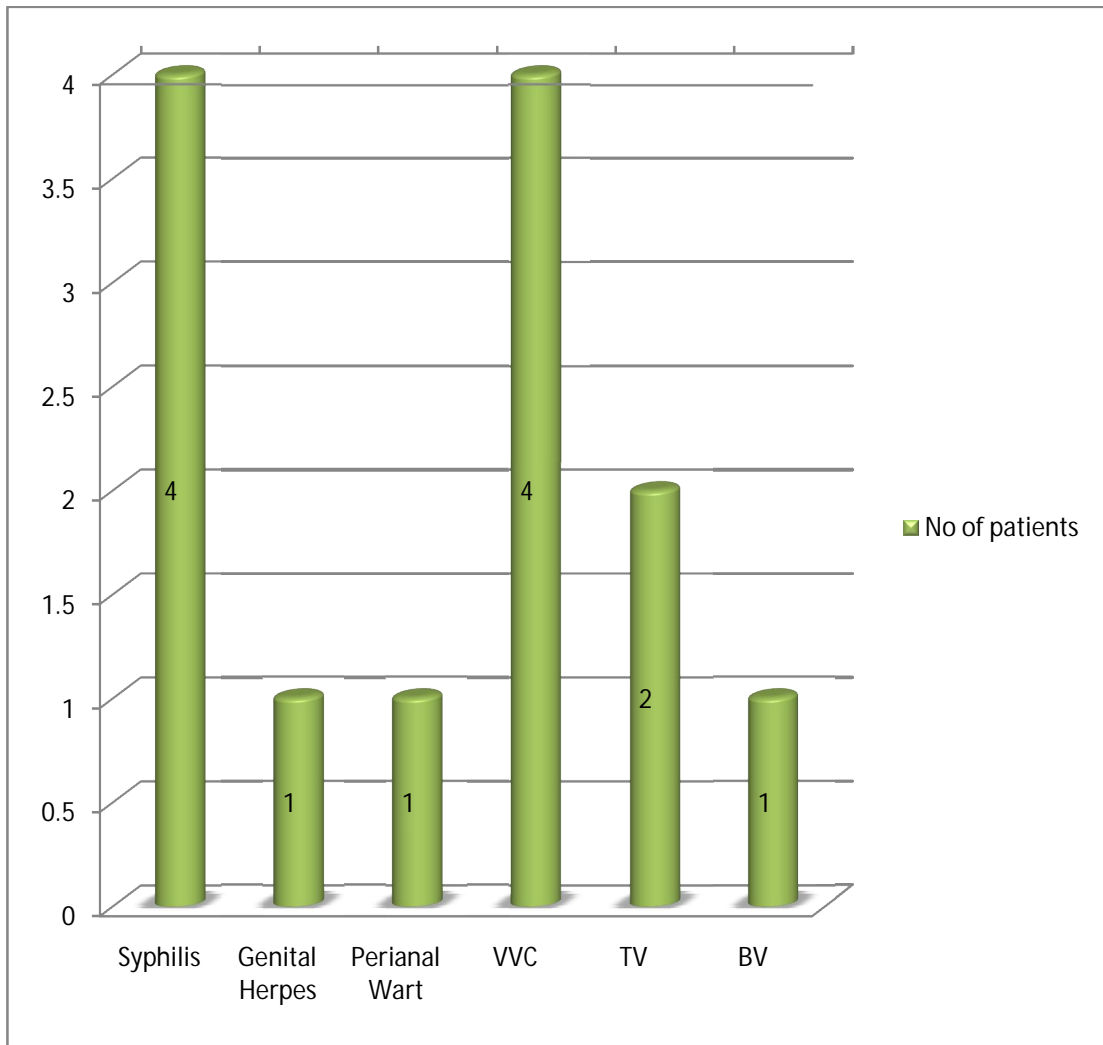
PERIANAL WART

GENITAL HERPES

ORAL CANDIDIASIS	TINEA CAPITIS

DERMATOLOGICAL MANIFESTATIONS

Oral candidiasis	14	28%
Pruritic papular dermatoses	4	8%
Extensive dermatophytoses	2	4%
Herpes zoster	3	6%
Folliculitis	4	8%
Angular cheilitis	3	6%
Tinea capitis	1	2%
Scrofuloderma	1	2%
Seborrheic dermatitis	2	4%
Exfoliative dermatitis	1	2%
Aphthous ulcer	1	2%

- Oral candidiasis was found to be the most common mucocutaneous manifestation (28%) .

- The other manifestations observed were folliculitis (8%), papular dermatoses (8%), herpes zoster (6%), angular cheilitis(6%), seborrheic dermatitis(2%), extensive dermatophytoses (4%), tinea capitis (2%), scrofuloderma(2%), exfoliative dermatitis (2%), aphthous ulcer (2%).

| DERMATOPHYTOSIS | ANGULAR CHEILITIS |
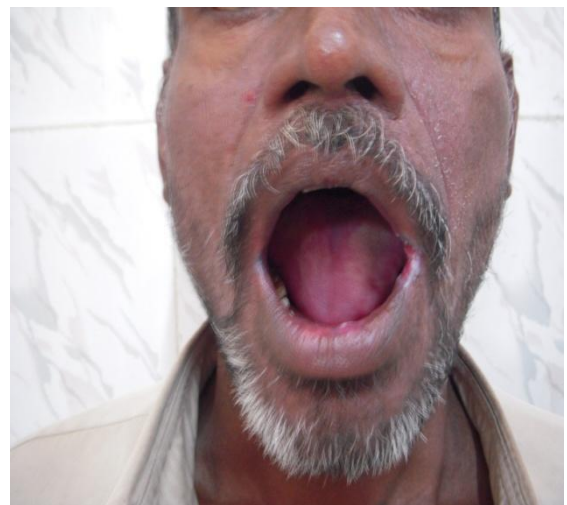
PRURITIC PAPULAR ERUPTIONS

SCROFULODERMA	HERPES ZOSTER
EXFOLIATIVE DERMATITIS	

SEBORRHEIC DERMATITIS

SUMMARY OF THE RESULTS

Most common age group affected	20-50 Yrs
Male: female ratio	29:20
Most common risk factor for HIV transmission	Heterosexual high risk behavior
Mean CD4 count	341cells/µL
CD4 count range	38-921 Cells/µL
Mean viral load	4.8×10^4 copies/ml
Viral load range	1×10^2 to 458×10^6 copies /ml.
Correlation between CD4 count and viral load	Correlation coefficient is -0.38 (negative correlation)

Discussion

DISCUSSION

This study was carried out to determine the clinical status, immunological status (CD4 count) and virological status at the time of presentation. In most of the supporting studies it was found that the progression of HIV infection to AIDS is determined by the basal viral load of the patients at the time of presentation. Thus in the present era of advanced molecular techniques , it becomes imperative to assess the virological status of the patients.

In this study a total of 50 treatment naïve HIV positive patients were randomly selected and assessed for clinical, immunological and virological status. The opportunistic infections and their relation to the CD4 count and viral load was analysed.

- In this study the most common risk factor was found to be heterosexual high risk behavior in concordance with other studies.

- Injection drug abuse was observed in 2 patients belonging to low socioeconomic status and educational status.

- In this study, the most common clinical presentation were found to be various dermatoses. Thus it is important to screen for HIV infection among the patients with dermatological conditions such

as pruritic papular eruption, oral candidiasis, widespread furunculosis , widespread dermatophytoses.

- Among the dermatological manifestations observed oral candidiasis was the most common, seen in 28% of the patients. It is observed that in various studies oropharyngeal candidiasis is the most common opportunistic infection occurring in upto 90% of the patients at some point of time during the course of HIV infection. It precedes most other opportunistic infections and it may be a sign of transition to AIDS. [57,58]

- One of the patients in this study had severe cutaneous drug reaction in the form of exfoliative dermatitis associated with fever, jaundice and elevated liver enzymes . From various studies it was found that the incidence of cutaneous drug reactions from a variety of drugs is high in HIV infection. These drug eruptions tend to be even more severe in HIV infected than in HIV uninfected patients.[59]

- In this study 19 patients who belonged to stage 1 disease were asymptomatic. Most of the patients were diagnosed to have HIV infection by partner screening. This stresses the importance of partner screening in the patients.

- In this 2 of the patients were asymptomatic for more than 8 years while their partners have progressed to AIDS and died. This can be explained by the fact that they may be long term non progressors. Their CD4 counts were 339, 351 and viral load was found to be 1,371,400.

- In this study highest number of patients were in stage 1 (19) and least in stage 4 (3). Thus it may be inferred that the number of patients diagnosed in the earlier stage of the disease has considerably increased because of increased awareness in the community about the disease and effective screening system available.

- The most common opportunistic infection in our study was oral candidiasis followed by tuberculosis. Tuberculosis was observed in patients with a CD4 count <500 cells / µl. Of the 10 patients who presented with tuberculosis, only 2 of the patients had a CD4 count between 350- 500 cells /µl. The remaining 8 patients had a CD4 count < 350 cells/ µl. 1 patient who had a CD4 count < 50 cells /µl presented with extrapulmonary tuberculosis. This is in concordance with a study in which tuberculosis was found to be most common in the CD4 count range of 200 -500 cells/µl.[60]

- 3 of the patients in stage 4 had a CD4 count below 100. All the 3 of them had AIDS associated wasting syndrome, 2 of them had oesophageal candidiasis and one of them presented with extrapulmonary tuberculosis. Thus there is a strong negative correlation between CD4 count and incidence of opportunistic infections.

- In this study baseline mean CD4 count was found to be 341 signifying severe immunosuppression. The range of CD4 count was found to be 38 – 921 cells / µl. A similar study done by Gautam et al in New Delhi showed a mean CD4 count of 140 cells/ µl.[61]

- In this study there was a significant difference in the CD4 counts

 $(p < 0.005)$ among patients belonging to the different stages. The CD4 count decreased with increasing stage of the disease. Natural history studies and clinical trials have shown that the CD4 count is an independent risk factor for progression to AIDS. It estimates the immunological status of the patient and it is an excellent marker of immediate risk for opportunistic infection.[62]

- It is also found that 90% of the patients had a CD4 count below 500 cells/ µl and infections occur with higher frequency in these

patients. 8 patients had CD4 count < 200 cells/µl which is a severe state of immunosuppression when a host of opportunistic infections occur.

- In this study viral copies determined by Real time PCR ranged from 1×10^2 copies to 4.58×10^6 copies / ml. The mean viral load was found to be 4.8×10^4 copies/ml. The plasma HIV RNA levels differed between different clinical stages. Mean viral load in stage 2 was found to be 2×10^3 copies/ml and stage 3 was found to be 11×10^3 copies/ml in contrast to stage 4 where it was found to be 1.2×10^5 copies/ml. There is a significant difference in the viral load between these stages

  ($p< 0.001$).

- WHO stage 4 patients (n=3), had circulating viral levels comparable to those observed during the peak viral replication in WHO stage 1

  (asymptomatic patients). In stage 4 it was found to be 1.2×10^5 copies/ml and in stage 1 it was found to be 9.5×10^4 copies/ml. (p value is 0.7117, not significant).

- High viral load in stage 1 can be explained by the fact that early part of stage 1 (1-2 years of infection) is the time during which the viral replication is maximum before the cell mediated immunity mounts an immune response and controls the viral replication. After the initial viral load peak there will be a steep decline in viral load within 2 years after the HIV infection to reach the steady state level. Many of our patients might have been diagnosed in the earliest part of HIV infection (before reaching Viral set point)

- High viral load in stage 4 can be explained by the fact that there is a complete loss of immunological control over viral replication and there is also a very low CD4 count at this stage.

- There was also significant correlation between increasing RT PCR determined HIV RNA levels and absolute CD4 counts .

  (correlation coefficient = - 0.3800) (p= 0.0065).

All these observations are in agreement with the study done by Piatak et al [63]

Conclusion

CONCLUSION

- In this study the most common risk factor elicited was heterosexual high risk behavior. Injection drug abuse and mother to child transmission was also observed.

- The most common presentation was various dermatoses followed by upper respiratory tract infections and cachexia.

- Oral candidiasis was the most common mucocutaneous manifestation observed.

- There was a strong correlation between increased frequency of opportunistic infections and low CD4 count.

- Tuberculosis was found to be more common when CD4 count is less than 350 cells /µl

- There was strong correlation between the worsening stage of the disease and decreasing CD4 count.

- Stage 1 HIV infection had very high viral load may be because most of the patients were in their earliest part of the Stage 1 disease. A relatively high CD 4 count was observed in these patients.

- Stage 4 showed a very high viral load and a very low CD 4 count which is well correlated with the pathogenesis of the disease.

- Stage 2 and 3 had significantly low viral load and a declining CD 4 count.

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Proforma

PROFORMA

1. PATIENT PROFILE

Patient ID No:

Date of registration:

Patient referred from: STD clinic / ART centre:

Name of the patient:

Age:

Sex:

Patients phone no:

Address :(Urban /Rural):

Education status:

Occupation:

If Patient diagnosed HIV positive in any other centre : YES/ NO

If yes where:

Purpose for referral:

2. HISTORY

Chief complaints:

History of presenting complaint:

3. MEDICAL HISTORY

HBV carrier: Yes ☐   **No** ☐   **Unknown** ☐

HCV Carrier:Yes ☐   **No** ☐   **Unknown** ☐

Other STI: Yes /No (specify if yes)

Diabetes ☐   **Hypertension** ☐   **cardiovascular disease** ☐

Present or past history of tuberculosis:

Current medications:

4.PERSONAL HISTORY	5.FAMILY HISTORY			
Risk factor for HIV	**Marital status:**			
☐ **Heterosexual**	Single ☐ married ☐ divorced ☐			
☐ **MSM**	Widow /widower ☐			
☐ **Injection drug use**				
☐ **Blood transfusion**	**Family members** **Partner/children**	**Age/sex**	**HIV** **status**	**ART** **Y/N**
☐ **Mother to child**				
☐ **Probable unsafe injection**				
☐ **Unknown**				
Habit of alcohol use:				
Habit of smoking:				
Use of condoms :				

6. CLINICAL EXAMINATION:

GENERAL EXAMINATION: PULSE   :   BP :

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM:

ABDOMEN:

CNS EXAMINATION:

GENITAL EXAMINATION:

DERMATOLOGICAL EXAMINATION:

7. TYPE OF TUBERCULOSIS AND TREATMENT TAKEN:

DISEASE CLASS		TB Regimen	
Pulmonary	☐	Category I	☐
Extra pulmonary	☐	Category II	☐
Past history	☐	Category III	☐

8.

dd/mm/yy	WHO clinical stage	Weight kg	Height cm	CD 4 count	Viral load

9.OTHER INVESTIGATIONS:

Investigations	Date	Values
Hb		
TLC		
DLC		
ESR		
PLT		
Serum creatinine		
Blood urea		
Blood sugar		
SGOT		
SGPT		
VDRL		
HBs Ag		
Chest X Ray		

Master Chart

Patients code	Age	Sex	Mari-tal status	Years since diagnosis	Clinical diagnosis at the time of presentation	Tuber-culosis status	HBV status Y/N	Risk fators	Partner/ children HIV status	STI'S	Dermatoses	WHO staging	CD 4 cell count	Viral load
P001	23	TG	S	1 Yr	Asymptomatic	No	No	homo			NIL	I	419	12388
P002	27	F	MR	1 month	Loss of weight,Oral candidiasis	No	No	hetero	H+ C-	VVC	Oral candidiasis	III	55	8542
P003	46	F	MR	2 Yrs	Asymptomatic	No	No	hetero	H+ C-		NIL	I	327	23129
P004	30	F	MR	1 Yr	malaise, anaemia	No	No	hetero	H+ C-		NIL	I	324	80252
P005	35	M	MR	2 yrs	PUO,Oral candidiasis	No	No	hetero	W- C-	Syphilis	Oral candidiasis, PPD	II	302	3359
P006	56	F	MR	3 yrs	Asymptomatic	No	No	hetero	H+ C-		NIL	I	705	23346
P007	35	F	W	2 Yrs	Asymptomatic	No	No	hetero	H+		NIL	I	368	67816
P008	43	M	MR	6 months	CVA,Oral candidiasis	No	No	hetero	UK		oral candidiasis	II	438	1973
P009	30	F	W	3 yrs	PUO, URI,Oral candidiasis	No	No	hetero	H+	VVC	oral candidiasis	II	488	2200
P010	43	M	MR	2 months	Tuberculosis, oral candidiasis,herpes zoster	Yes	No	hetero	W- C-		oral candidiasis, herpes zoster	III	131	31203
P011	39	M	MR	8 months	Asymptomatic	No	No	hetero	W+ C-	Syphilis	NIL	I	390	23002
P012	35	M	MR	1 yr	hepatitis B, Tuberculosis,anaemia	Yes	Yes	hetero	W+ C+		NIL	III	68	9243
P013	23	F	W	6 yrs	Asymptomatic	No	No	hetero	H+ C-		NIL	I	921	41344
P014	27	M	S	3 Yrs	PUO, Diarrhoea,Oral candidiasis	No	No	hetero			Oral candidiasis	III	447	2334
P015	33	F	MR	7 yrs	Asymptomatic	No	No	hetero	H + C+	VVC	NIL	I	447	77231
P016	27	F	MR	8 yrs	Asymptomatic	No	No	hetero	H +		NIL	I	519	3463
P017	32	M	MR	1 yr	pruritic papules	No	No	hetero,iv	W- C-		PPD	II	505	212
P018	34	M	MR	1mth	Asymptomatic	No	No	hetero,iv	W-		NIL	I	226	372033
P019	30	M	MR	2yrs	Tuberculosis,Oral candidiasis	Yes	No	hetero	W-		Oral candidiasis	III	386	1276
P20	29	F	MR	3 yrs	Asymptomatic	No	No	hetero	H+C+		NIL	I	368	458492
P021	38	M	MR	7 mths	fever,hepatitis B,Oral candidiasis	No	Yes	hetero	W-C-		oral candidiasis, PPD	III	211	29842
P022	47	M	S	6 yrs	folliculitis	No	Yes	hetero			folliculitis	II	356	3459
P023	30	M	MR	2 yrs	tuberculosis,anaemia	Yes	No	hetero	W+		herpes zoster	III	443	1370
P024	54	M	MR	8 days	extensive dermatophytosis	No	No	hetero	W+		angular cheilitis, dermatophytosis	III	268	1263

Patients code	Age	Sex	Marital status	Years since diagnosis	Clinical diagnosis at the time of presentation	Tuberculosis status	HBV status Y/N	Risk fators	Partner/ children HIV status	STI'S	Dermatoses	WHO staging	CD 4 cell count	Viral load
P025	14	M	S	3 mths	tinea capitis,fever	No	No	MTCT	M+		pustular tinea capitis	III	364	16711
P026	31	F	MR	3 yrs	Asymptomatic	No	No	hetero	H+C+	VVC	NIL	I	308	30034
P027	51	M	MR	7yrs	herpes zoster,candidiasis	No	No	hetero	W+C-		Oral candidiasis	III	339	3331
P028	49	F	MR	3yrs	itching all over the body	No	No	hetero	H+		NIL	I	459	18990
P029	34	M	MR	new	herpes genitalis,latent syphilis,tuberculosis ,weight loss	Yes	No	hetero	Uk	Syphilis	herpes genitalis, scrofuloderma	III	333	4457
P030	48	M	MR	new	oesophageal candidiasis,weight loss,anaemia,extra pulmonary tuberculosis	Yes	Yes	hetero	UK		NIL	IV	38	197432
P031	31	M	MR	new	sycosis barbae	No	No	hetero	W-		sycosis barbae	II	412	1183
P032	30	F	MR	new	Asymptomatic	No	No	hetero	H+		NIL	I	503	12542
P033	48	M	MR	3mths	fever,oral candidiasis	Yes	Yes	hetero	W+		oral candidiasis	III	301	11932
P034	40	M	MR	new	recurrent fever,seborrheic dermatitis,angular cheilitis,tuberculosis	Yes	No	hetero	UK		angular cheilitis, seborrheic dermatitis	III	254	100
P035	38	M	MR	new	recurrent fever,tuberculosis,chronic diarrhoea,Oral candidiasis,herpes zoster	Yes	No	hetero	W+		oral candidiasis,herpes zoster	III	184	7112
P036	41	F	W	new	Asymptomatic	No	No	hetero	H+C-	TV	NIL	I	223	142971
P037	38	M	MR	new	wasting syndrome,prolonged fever,oral aphthae,prolonged diarrhoea,Oral candidiasis	No	No	hetero	W+C-		Oral Aphthae, Oral candidiasis	IV	92	49025
P038	30	M	S	new	Asthenia,Furunculosis,dermatophytoses	No	No	hetero			Furunculosis, dermatophytosis	II	342	1114
P039	42	M	MR	new	Wasting syndrome,oesophageal candidiasis	No	No	hetero	W+		angular cheilitis,oral candidiasis,pallor	IV	53	131315
P040	36	F	MR	new	Asymptomatic	No	No	hetero	H+	Syphilis	NIL	I	309	43671
P041	44	M	MR	new	perianal wart	No	Yes	bisexual	W+		Perianal wart	II	220	7114

Patients code	Age	Sex	Mari-tal status	Years since diagnosis	Clinical diagnosis at the time of presentation	Tuber-culosis status	HBV status Y/N	Risk fators	Partner/ children HIV status	STI'S	Dermatoses	WHO staging	CD 4 cell count	Viral load
P042	44	F	W	new	Prolonged fever,tuberculosis exfoliative dermatitis	Yes	No	hetero	H+	TV	Generalised pruritus, exfoliative dermatitis	III	65	29022
P043	40	M	MR	new	Tuberculosis,papular pruritic eruption,Fever	Yes	No	hetero	W+C-uk		PPD	III	296	40021
P044	55	F	MR	new	Prolonged diarrhoea,prolonged fever,Weight loss	No	No	hetero	H+	BV	diffuse pigmentation of skin	III	493	1459
P045	55	F	MR	new	Weight loss	No	No	hetero	H+C-		Generalised pruritus	II	492	3981
P046	23	F	MR	new	Asymptomatic	No	No	hetero	H+C-		NIL	I	466	12899
P047	46	F	MR	2 Yrs	Asymptomatic	No	No	hetero	H+ C-		NIL	I	327	38862
P048	39	M	MR	8 months	Asymptomatic	No	No	hetero	W+ C-		NIL	I	390	331241
P049	47	M	S	6 yrs	folliculitis	No	Yes	hetero			folliculitis	II	356	2345
P050	51	M	MR	7yrs	herpes zoster,candidiasis	No	No	hetero	W+C-		Oral candidiasis	III	339	3331

KEY TO MASTER CHART

1. M : Male

2. F : Female

3. TG : Transgender

4. Hetero: Heterosexual

5. Homo: Homosexual

6. S:Single

7. MR: Married

8. Yrs: Years

9. Mths: Months

10. PUO: Pyrexia of unknown origin

11. URI: Upper respiratory tract infection

12. CVA: Cerebro vascular accident

13. W+ : Wife is tested positive for HIV infection

14. H+ : Husband is tested positive for HIV infection

15. C+ : Child is tested positive for HIV infection

16. W- : Wife is tested negative for HIV infection

17. H- : Husband is tested negative for HIV infection

18. C- : Child is tested negative for HIV infection.

19. IV : Intravenous drug abuse

20. PPD: Pruritic papular dermatoses

21. W: Widow

22. UK- Unknown

23. M+ : Mother is tested positive for HIV infection

24. MTCT : mother to child transmission

25. STI'S : Sexually transmitted infections

26. VVC: Vulvovaginal candidiasis

27. BV: Bacterial vaginosis

28. TV: Trichomonas vaginalis vaginitis

PATIENT CONSENT FORM

Title of the study   : **"A Real-Time PCR based study on HIV viral load and its correlation with the clinical and immunological status of the HIV positive patients"**

Name of the participant: …………………………………………………………

Name of the Primary Investigator: Dr.G.Rajesh Kumar

Name of the institution: Institute of Venereology

Documentation of the informed consent:

I ……………………………………………….have read the information in this form

I was free to ask any questions and they have been answered

To be included as a participant in the above study.

1. I have read and under stood this consent form and the information provided.

2. I have had the consent document explained to me.

3. I have been explained about the nature of the study.

4. The rights and responsibilities have been explained to me by the investigator.

5. I am aware of the fact that I can opt out of the study at any time

6. I hereby give permission to the investigators to release the information obtained as a result of participation in this study to the sponsors, regulatory authorities,Govt.Agencies and IEC. I understand that they are publicly presented.

7. My identity will be kept confidential if those data are publicly presented.

8. By signing this consent form I attest that the information given in this document and the HIV

consent form have been clearly explained to me .

Date: Signature of the patient