"CORRELATION BETWEEN CD4 COUNT AND TOTAL LYMPHOCYTE COUNT WITH RESPECT TO CLINICAL PROFILE OF NEWLY DIAGNOSED HIV POSITIVE PATIENTS"

By

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Dissertation submitted to

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

TAMAKA, KOLAR, KARNATAKA

In partial fulfilment of the requirements for the Degree of

M.D.

In

GENERAL MEDICINE

Under the guidance of

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APRIL 2012

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I hereby declare that this dissertation entitled

"CORRELATION BETWEEN CD4 COUNT AND TOTAL LYMPHOCYTE COUNT WITH RESPECT TO CLINICAL PROFILE OF NEWLY DIAGNOSED HIV POSITIVE PATIENTS"

is a bonafide and genuine research work carried out by me under the guidance of

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Date:

LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
CD4	Cluster of Differentiation 4
TLyC	Total Lymphocyte Count
CDC	Centre for Disease Control and Prevention
AZT	Zidovudine
HTLV-BLV	Human T-cell lymphotropic virus/bovine leukemia virus
RNA	Ribonucleic Acid
LTR	Long Terminal Repeat
DNA	Deoxy Ribonucleic Acid
MHC	Major Histocompatibility Complex
ARC	AIDS Related Complex
WHO	World Health organization
UNAIDS	United Nations Program on AIDS
STIs	Sexually Transmitted Illnesses
ELISA	Enzyme Linked Immunoassays
NAAT	Nucleic Acid Amplification Technique
IFA	Immunofluorescent assay
LIA	Lineimmuno assay
RIPA	Radioimmuno precipitation assay
PCR	Polymerase Chain Reaction
NASBA	Nucleic acid sequence based amplification
WB	Western Blot
ART	Anti-Retro Viral Therapy
HAART	Highly Active Anti-Retro Viral Therapy
CSF	Cerebrospinal Fluid
Hb	Hemoglobin
CNS	Central nervous system
OP	Organophosphate
FNAC	Fine Needle Aspiration Cytology
TLC	Total Leucocyte Count
GI	Gastro-intestinal
CT	Computerized Tomography
ESR	Erythrocyte Sedimentation Rate
TB	Tuberculosis
PCP	Pneumocystis jerovecii Pneumonia
CM	Cryptococcal Meningitis
MAC	Mycobacterium Avium Complex
CMV	Cytomegalo Virus

ABSTRACT

Aims and Objectives: To study the clinical profile of HIV infected patient. To understand the high risk behaviours involved in the transmission of the virus. To study the correlation between Total Lymphocyte Counts (TLyC) and CD4 counts in HIV infected patient.

Methods: 50 consecutive cases of HIV positive confirmed by ELISA attending Sri R. L. Jalappa Hospital are included in the study. Selected patients undergo careful history recording and general physical and systemic examination. This is followed by routine investigations which include complete hemogram with peripheral smear, urine routine and microscopy, stool examination, chest X-ray, blood sugar, blood urea, serum creatinine, CD4 cell count, TLyC and others. Some special investigations like ultrasonography (USG) of abdomen, CT, MRI, serous fluid analysis will be carried out as the clinical condition warrants.

The obtained data will be subjected to descriptive statistical analysis and results will be based upon the data analysed.

Results:HIV predominantly affected the sexually active group between 20-49 years with male preponderance and was mainly transmitted sexually through unsafe sexual practices. Females had higher mean CD4 cell count (419 cells/cumm) and mean TLyC (1783.79 cells/cumm). Patient groups with CNS manifestations, lymphadenopathy, significant chest X-ray findings had significant correlation between TLyCand CD4 cell count.

Overall, at CD4 cell count <200 cells/ cumm, the TLyC correlated significantly with CD4 cell count ($r = 0.4757$, $p = 0.0005$).

Conclusion: This study shows that there is strong positive correlation between CD4 cell count (<200 cells/cumm) and TLyC. TLyC can be employed as a suitable surrogate marker to CD4 cell count in monitoring HIV individuals in resource constrained settings with advanced state of immune suppression defined both clinically and by laboratory measures.

Key Words: HIV, AIDS, CD4 cell count, Total Lymphocyte Count, Surrogate markers

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INTRODUCTION

"The AIDS epidemic has rolled back a big rotting log and revealed all the squirming life underneath it, since it involves, all at once, the main themes of our existence: sex, death, power, money, love, hate, disease and panic." **Edmund White**

"The global HIV/AIDS epidemic is an unprecedented crisis that requires an unprecedented response. In particular it requires solidarity -- between the healthy and the sick, between rich and poor, and above all, between richer and poorer nations. We have 30 million orphans already. How many more do we have to get, to wake up?" **Kofi Annan**

These are the words of some of the eminent people of the society regarding the growing concern over the pandemic of AIDS that has stuck fear in the minds of millions; both affected by the virus itself and those healthy. AIDS represents the model of all catastrophes attacking and weakening the immune system of the country. Today it represents large part of the awareness for what it represents.

Despite the increasing efforts by the government and non-governmental organization, this pandemic is still far from control and efforts to cure it remain a dream to achieve. The drugs available in the market can only prolong the life of the affected individuals, but in reality it just postpones ones sufferings. The extensive research has still not explained the disease course completely and monitoring response to therapy. Further, predicting the occurrence of complications based on suitable laboratory markers is a challenge to research scholars and clinicians.

The problem is even more severe in the developing countries like India with high prevalence rates of AIDS, where high cost curtails frequent monitoring of the patients. The result, timely intervention gets delayed. Research is undergoing to identify a suitable low cost surrogate marker to CD4 cell count to predict the immune status of AIDS related

complications. Till date CD4 count estimation has been used as a marker to monitor disease progression, predict the occurrence of complications and monitor treatment response. But the expensive nature of this investigation curtails regular monitoring of the patients. The total lymphocyte count (TLyC) estimation which is easily available and less expensive investigation at the peripheral center is now being investigated for its accuracy in predicting the immune status of the HIV positive individuals and treatment outcome. This study is undertaken to identify the correlation between TLyC and CD4 count with respect to clinical profile of newly diagnosed HIV positive patients.

AIMS AND OBJECTIVES

To study the clinical profile of HIV infected patient. To understand the high risk behaviours involved in the transmission of the virus. To study the correlation between Total Lymphocyte Counts (TLyC) and CD4 counts in HIV infected patient.

REVIEW OF LITERATURE

It's been 3 decades since the first description of the Human Immunodeficiency Virus (HIV), the global fight against this devastating infection still continues. Many governmental and non-governmental organizations are actively committed in research and other activities to better understand HIV and how it causes disease, find new and effective tools to diagnose, monitor and prevent the transmission of the virus, develop more effective treatment and possibly find a cure.

According to Morbidity and Mortality Weekly Report, august 11, 2006, an estimated 65 million people are infected with HIV worldwide and 25 million deaths have been reported.[1]It is sometimes difficult to recall how it all started and how we arrived at this stage of the disaster facing one of the most serious threats of public health.

1. The Beginning

Tracing the history of the virus is like any other mystery. Although today there has been a debate of the existence of the virus much before 1980's but the HIV came to the world's conscience in the early summer of 1981 in Los Angeles, California with the description of a rare pneumonia caused by Pneumocystis carinii (now known as P.jiroveci) in 5 young homosexual men.[2]At about the same time physicians in New York city reported CDC of a severe form of Kaposi's Sarcoma in 26 young patients.[3] All these patients had immunodeficiency of unknown reason and with growing knowledge many more such reporting were documented. An epidemic of growing concern was alarming the health officials and CDC launched a nationwide surveillance program. Soon physician's reporting of such acquired immunodeficiency with opportunistic infections and cancers started coming from across the globe. The pandemic was growing at an alarming rate. CDC coined the new disease as Acquired Immunodeficiency Syndrome (AIDS).

After several attempts in identifying the causative agent, the first break through was achieved in 1983, when scientists in France discovered the causative agent to be a virus that was totally new.[4] Soon to be followed were the virologists from United Sates of America (USA), who in 1984 published their report confirming the virus as the causative agent.[5]

By 1985, serological tests were developed to detect HIV infection in asymptomatic individuals, detect seroconversions and screen blood products.[6]The year 1987, saw the dawn of first defensive attack against the HIV with the availability of zidovudine (AZT) for treatment.[7]

The growing concern and awareness led to increasing research and further breakthrough in the historical battle against the ravaging agent. Many important milestones have been achieved but still man has not been victorious to wipe off the virus completely.

Understanding the virus, its architecture and pathophysiology involved in its survival, transmission and disease process tells why it is not possible to find a cure from it completely.

2. The Human Immunodeficiency Virus

HIV virus belongs to a large family of RNA viruses, the Retroviridae. The Lentiviruses and HTLV-BLV virus groups of retroviridae are of particular importance as they cause human disease. HIV – 1 and HIV – 2 are the important members of the Lentivirus genus.

2.1 Structure of HIV

Both HIV-1 and HIV-2 are morphologically similar spherical structures of 120 nm in diameter consisting of an outer envelope of lipid bilayer with glycoprotein molecules protruding from it.[8- 10] Enclosed within is the icosahedral shell of matrix protein within which is the vase-shaped conical (helical) capsid.[8- 10] The capsid encases the viral RNA.[8-10] The vase and genome together form the nucleocapsid.

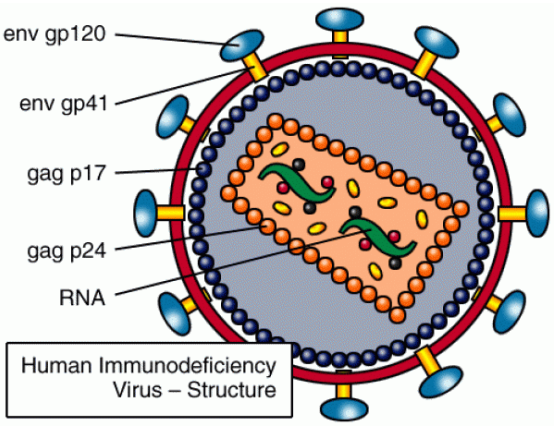

Fig.1: Structure of HIV

2.2 HIV genome[8,9]

HIV consists of 2 identical copies of single stranded RNA, both with positive polarity; hence the genome of HIV is diploid. The viral RNA is composed of at least nine different genes, of which the major three (gag, pol and env) are common to all retroviruses. These genes encode genetic information that allows viruses to make either structural or regulatory proteins during replication.

The gene composition of HIV has been mapped and is shown in Fig.2

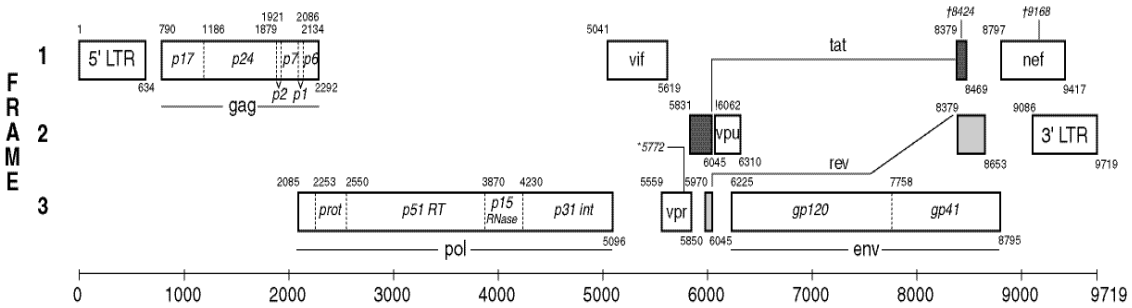

Fig.2: Gene Mapping of HIV

A brief summary of the genes encoding various viral proteins is shown in Table.1.

Table 1: HIV Genes

THE GENES	Types of proteins Encoded	Proteins Encoded
env gene	Surface glycoprotein	gp41, gp120
gag gene	Nucleocapsid and core proteins	p6, p7, p17, p24
pol gene	Viral enzymes	reverse transcriptase (p66/p51), RNaseH, protease (p10) and integrase (p32)
tat gene	Early regulatory protein	p14, increase viral replication
rev gene	Early regulatory protein	p19, increase viral replication
Nef gene	Early regulatory protein	p27, enhances HIV infectivity
Vif gene	Late regulatory proteins	p23, efficient release of budding virions from the cell
Vpu gene	Late regulatory proteins	p15, efficient release of budding virions from the cell (only in HIV-1 but not in HIV-2)
Vpr gene	Accessory proteins	Important for efficient infection and viral replication in the natural target cells
Vpx gene	Accessory proteins	Found only in HIV-2

The genomes of all retroviruses contain long terminal repeat (LTR) elements, generated during reverse transcription and only completely present in the corresponding DNA copy of the viral genome. These LTRs do not encode for protein, but are essential binding sites for initiation of viral transcription and the regulation of viral gene expression.

2.3 Core proteins, glycoproteins and the viral envelope[8,9]

Core proteins is double protein coat, the outermost layer of which is a icosahedral shell containing an inner helical cone shaped vase that encloses the viral genome. The vase is constructed of a capsid protein, p24 and the icosahedral shell consists of matrix protein, p17.

The genome also contains two nucleocapsid proteins; p6 (plays a role in virion assembly and release) and p7 (a binding protein for the two single strands of RNA).

2.4 Viral enzymes

These include the important ones like reverse transcriptase, integrase and protease.

2.5 Lipid bilayer and envelope glycoproteins[8,9]

The lipid bilayer which surrounds the core proteins has important glycoprotein structures attached to and embedded in it. The important ones are surface glycoprotein, gp120SU and transmembrane glycoprotein, gp41 TM.

This lipid bilayer derived from host cells carries host antigens on its surface and is studded with cellular proteins, including β2-microglobin and MHC class I and II molecules.

3. Virus Replication

The HIV infects and eliminates key cells of the immune system, thus rendering the body defenseless against cancer and infection. The virus infects variety of immune cells such as CD4+ T cells, macrophages, microglia and others (Table 2). This tropism of the virus is mediated through interaction of the virion surface glycoprotein gp 120 with the CD4 molecule and specific chemokine co-receptors on the target cells.[11]

Table.2 Primary cellular Targets for HIV[12-15]

- CD4+ T-lymphocyte
- B-Lymphocyte
- Natural Killer(NK) lymphocytes
- Endothelial cells
- Hematopoietic stem cells
- CD4+ blood monocytes and tissue macrophages (tissue histiocytes), including microglial cells in brain
- Epithelial CD4+ dendritic cells (Langerhans cells)
- Follicular dendritic cells in germinal centres of lymph nodes
- M-cells in Peyer's patches in gut
- Cerebroside cells in nervous tissue (brain) and gastrointestinal epithelial cells

The HIV replication cycle is discussed as below;

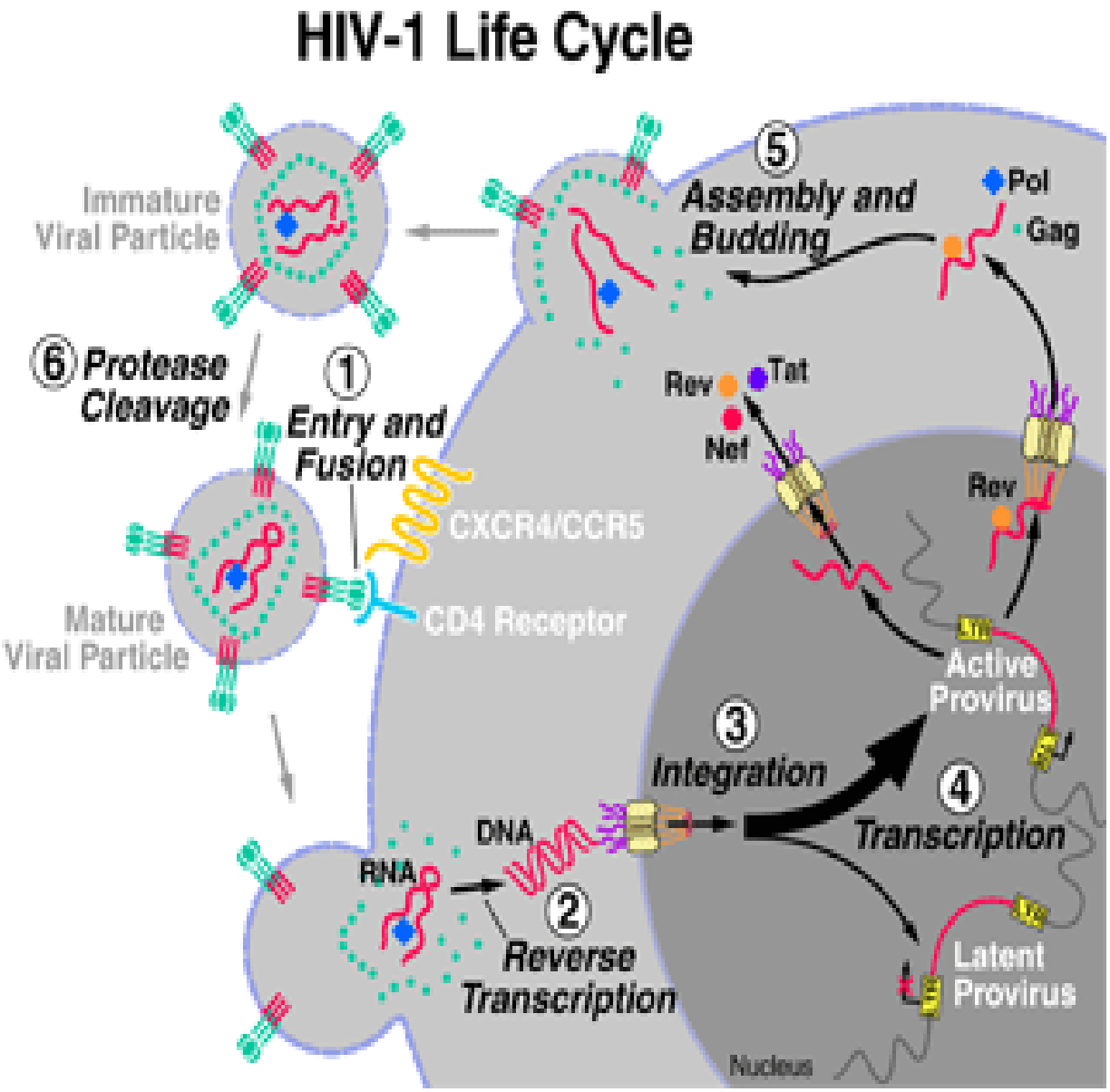

Fig.3: HIV Replication Cycle

3.1 Attachment

After gaining access into the human blood stream, the virus circulates throughout the body and specifically infects CD4+ cells. This is mediated by a process of interaction between surface glycoprotein, gp120 of the virus and the cell surface marker protein, CD4 of the target cells. Macrophages are the first in the cell line to get infected.

3.2 Entry into Macrophages and T-lymphocytes[8, 16-20]

After docking of gp120 onto the CD4 receptor, the virus requires a co-receptor on the macrophage, CCR5, to pull the virus across cell membrane.[16, 17, 20]The initial interaction between gp120-CD4, a conformational change takes place allowing the co-receptor to pass the gp12-CD4 complex through the cell membrane, triggering the passage of HIV contents into the cell by endocytosis.

Once infected the macrophages, the HIV undergoes constant replication and mutation. By chance mutation, the virus alters the gene for gp120 in a way that changes the allegiance of its co-receptor. The new form of gp120 now binds to a different co-receptor; CXCR4 present on the surface of CD4+ T-Lymphocytes.[18-20] Once the damage to the body's immune system is significant, it herald's the onset of AIDS.

3.3 Replication and Transcription[9, 21-23]

Soon after the capsid enters the cell, it loses its coat and releases the single stranded (+) RNA genome and the enzyme reverse transcriptase. This reverse transcriptase produces the antisense complementary DNA molecule using the single stranded RNA as template. Additionally, it has a ribonuclease and DNA-dependent DNA polymerase activity. It then creates sense DNA from the antisense cloned DNA, and finally forming the dsDNA molecule which is then transported to the host cell nucleus. Integration of viral DNA into the host cell genome is the carried by the enzyme integrase.

The integrated DNA provirus is then transcribed into mRNA, which is then spliced into smaller pieces and exported to cytoplasm. These small mRNA pieces are then translated to produce regulatory, structural and many other proteins essential for further viral replication and release after assembly into virion particles.

3.4 Assembly and Release[8,9]

This final step occurs at the plasma membrane of the host cell. This is common all the cells infected with the HIV within which the virus undergoes replication.

The env ploy protein (gp 160) is transported to the golgi apparatus through the endoplasmic reticulum and cleaved and processed into gp41 and gp120 glycoproteins. These are then transported to the plasma membrane. Other polyproteins are also associated with the inner surface of the plasma membrane. The HIV genomic RNA starts to assemble with the polyproteins and other enzymes as the virion starts budding from the host cell. The process of maturation into a mature HIV virion continues and once complete infects another cell.

4. Pathogenesis

"In this global emergency, prevention of HIV infection must be our greatest worldwide public health priority. Science will one day triumph over AIDS, just as it did over smallpox. Curbing the spread of HIV will be the first step. Until then, reason, solidarity, political will and courage must be our partners."

The Durban Declaration 2000 South Africa[24]

Now at the end of third decade of our experience with HIV, this global pandemic continues to perpetuate and infect millions across the world each year. This is due to the different ways in which the virus is transmitted and understanding these means is vital to devising preventive strategies.

4.1 Domains of Exposure[25-28]

HIV is a blood – borne virus. It has been isolated from blood, semen, pre-ejaculatory fluid, saliva, tears, breast milk, and cerebrospinal fluid and many other body fluids.

The different portals of exposure to this virus are shown in the figure below;

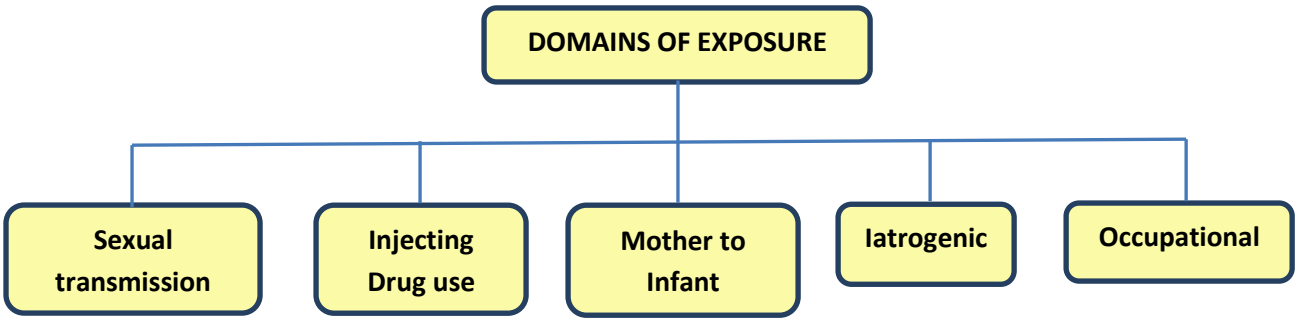

Fig. 4: Domains of exposure

Although sexual mode of exposure is the commonest route of becoming infected with HIV, there are varied and changing patterns of risk events and behaviors in

different regions, localities within the region and even in different populations within a community. For instance, while intravenous drug abuse may be most prevalent in one community becoming an important route of HIV transmission, the same may not be true for another community. Another example is vertical (mother to infant) transmission which is uncommon in developed countries but frequent in resource limited developing countries.

4.1.1 Sexual Transmission

HIV transmission can occur in both homosexual and heterosexual encounters. Unprotected penetrative heterosexual vaginal intercourse with an HIV infected partner is the most common means by which vast majority of people become infected with HIV. HIV is present in semen, pre-ejaculatory fluid, vaginal and cervical secretions, and in saliva.

In sub – Saharan Africa, Carribean, most of the Asia, Latin America, the south – east Mediterranean, and Western Europe, HIV is principally spread heterosexually. There are several risk factors that increase the likelihood of spread of HIV amongst the uninfected during sexual intercourse.

Table 3. Factors that increase the risk of HIV transmission during sexual intercourse[31-38]

- Likelihood that sexual partner is infected with HIV
- Type of sexual activity
- Disease stage of the infected partner
- Presence of other Sexually Transmitted Illnesses
- Frequency of partner change
- Biological factors
- Lack of male circumcision
- Viral variants
- Host susceptibility

4.1.2 Injecting Drug Use

About one third of all the cases of HIV transmission in the developed countries of North America and Europe are due to injecting drugs. The infection is efficiently transmitted by sharing contaminated needles, syringes and injecting paraphernalia.[38] Also, the injectable drug users are sexually active and indulge in high risk sexual activity which further enhances their risk of HIV acquisition.[40] Lastly, many of these individuals are homeless, unemployed, and downtrodden and live in poverty making it difficult for them to access timely preventive and healthcare services.

4.1.3 Mother – to – Infant (Perinatal, Vertical) Transmission

First reported in 1982, currently millions of children have become infected this way. Infants born to infected women can acquire HIV during pregnancy, childbirth or during breast feeding.[40]Any factor that predisposes the women to become sexually infected clearly increases the risk of vertical transmission. The disease stage and viral load are important considerations while considering this mode of transmission.Maternal-fetal HIV-1 transmission is multifactorial, with increased risk associated both with ICD p24 antigenemia at term and with intrapartum events that increase fetal exposure to maternal blood.[41]

Infants born to newly infected mothers or those with symptomatic disease or having high viral load are at greatest risk of becoming vertically transmitted.

4.1.4 Iatrogenic Transmission

Once a significant risk factor for HIV transmission, now the individuals becoming infected via iatrogenic transmission are on the decline especially in developed world due to improved medical care. But still the fear of transmission continues to haunt the public and this perceptional risk is out of proportion to the actual risk. The figure below shows some iatrogenic means of HIV transmission.

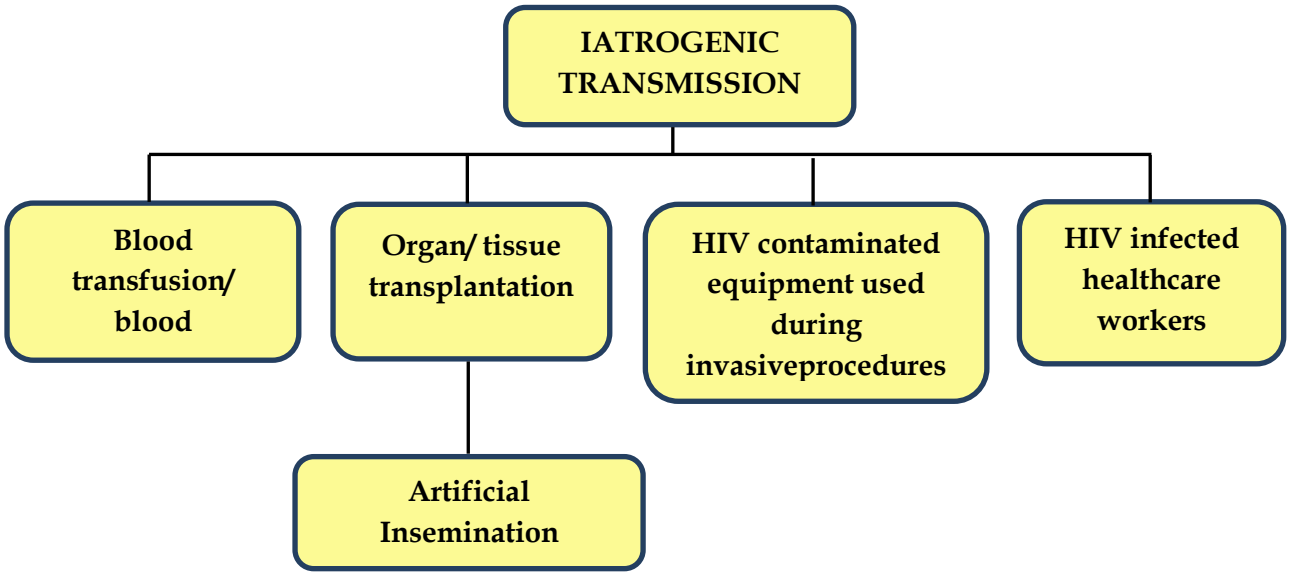

Fig.5. Iatrogenic Transmission of HIV

4.2 Stages of HIV – related Disease

The typical course of an untreated HIV infection spans about a decade. HIV infection produces a varied clinical spectrum ranging from a mild 'flu – like' illness to full blown AIDS. The course of infection with HIV-1 in HIV-infected humans may vary dramatically, even if the primary infections arose from the same source (Liu 1997).[42]The stages of progression of HIV infection to full blown AIDS is presented in the figure below;

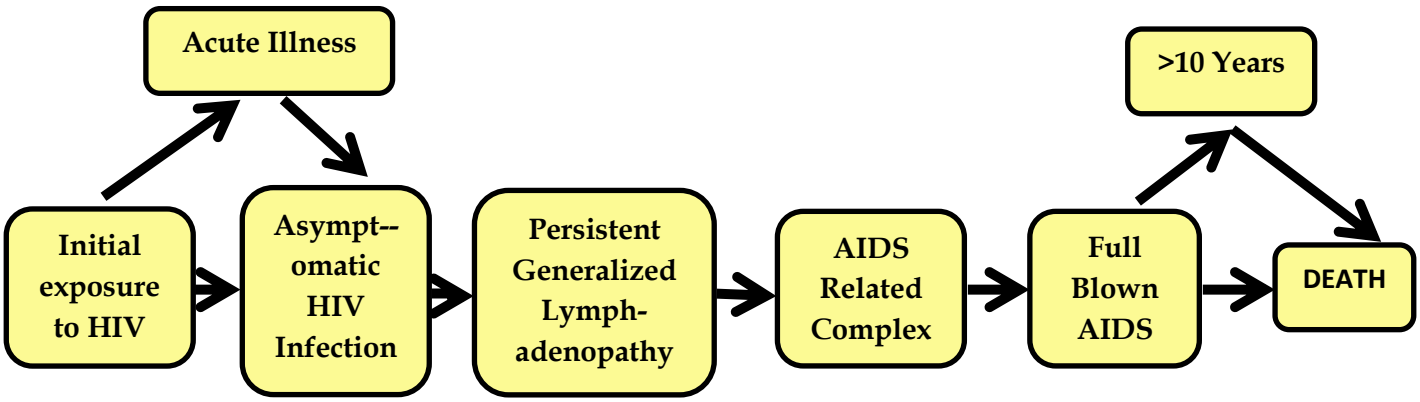

Fig.6. Stages of HIV Infection

4.2.1 Acute HIV Infection

About three to six weeks after exposure to HIV, individuals develop an acute 'flu – like' illness characterized by fever, sore throat, joint and muscle pains and other non-specific symptoms.[43] Sometimes, it is so mild that it passes off without the individual's knowledge. Anti – HIV antibodies develop by the end of this phase. This phase is also called as seroconversions illness.

4.2.2 Asymptomatic Stage

Individuals in this stage are usually healthy without any symptoms and signs of illness. However, they are potential virus carriers and can infect others through their blood and sexual fluids.

4.2.3 Persistent Generalized Lymphadenopathy

During this stage individuals have enlarged lymph nodes in the region of neck, axilla and groin.[45]Except this they are usually asymptomatic. This occurs due to widespread dissemination of the HIV into body especially the lymphoid organs where they actively replicate. The lymph node enlargement is a defensive response against the virus.

4.2.4 AIDS Related Complex (ARC)

Individuals in this stage present with constitutional signs and symptoms. This stage has varied clinical presentations and in the era where laboratory diagnosis was not available ARC was considered significant to diagnose AIDS. ARC is diagnosed in a patient who has unexplained low grade fever with or without night sweats lasting for more than 3 months with >10% weight loss or individuals who have persistent diarrhea. Despite of the above manifestations it is difficult to diagnose AIDS on clinical grounds alone especially in developing countries where there are many causes for above clinical presentations.

4.2.5 Acquired Immunodeficiency Syndrome (AIDS)

The last of HIV disease spectrum, here the patients are symptomatic either due to the opportunistic infections or cancers caused due to immune suppression by the HIV. In this stage there is significant decline in the CD4+ T-helper cells which play a key role in cell mediated immunity.

Thus, HIV infection is a chronic disease whose disease progression and clinical outcome depends on several factors, including host.

4.3 Clinical Features[45,46]

The continuing damage of immune system following HIV infection leads to dire clinical outcomes in infected individuals. The clinical manifestations following infection with HIV show geographic variations. For instance, Pneumocystis jirovecii pneumonia is common cause for pulmonary manifestations in developed countries while in developing countries bacterial pneumonias are most frequent causes mainly tuberculosis. Environmental conditions, mode of exposure, dosage of virus, gender, age, general well being and immune status of the infected individual all determine the clinical presentation and survival in infected individuals.

Clinical manifestations may be minimal or none but when present result from immunopathic, lymphocytopathic and neuropathic effects of HIV. Symptoms of acute HIV infection are non-specific and include fatigue, rash, headache, nausea, and night sweats. As the disease advances more serious symptoms begin to appear and are often preceded by a prodrome of illnesses like fatigue, malaise, weight loss, and fever, shortness of breath, chronic diarrhea, white patches on the tongue (hairy leukoplakia, oral candidiasis) and lymphadenopathy. No organ is immune following HIV infection and patient can present with any combination of symptom complex.

Skin diseases are the first and most common clinical presentation for which patients seek medical care. Lung is the most commonly affected organ and gastrointestinal manifestation are frequent cause of major debility in the patients.

Over the decades extensive research on AIDS has provided the healthcare workers with a comprehensive understanding of natural history of HIV infection beginning from initial infection through to end stage disease to death. This sequence has

been summed up into 3 distinct stages that have been encompassed in the Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adults and Adolescents, published in 1993 by the Centers for Disease Control and Prevention (CDC). First published in 1986 this system has been revised and republished in 1993. Another system WHO Clinical Staging of HIV/AIDS and case definition for the resource constrained developing countries was developed by World Health Organization (WHO) and the Joint United Nations Program on AIDS (UNAIDS). First published in 1990 this staging system has been revised in 2007.

4.3.1 CDC Classification System for HIV/AIDS[47-49]

The revised CDC classification system for HIV-infected adults and adolescents categorizes people on the basis of clinical conditions associated with HIV infection and CD4+ T-lymphocyte cell counts. The system is based on three ranges of CD4+ counts and three clinical categories and is represented by nine mutually exclusive categories. It is different from the previous classification system which was developed before the widespread use of CD4+ T-lymphocyte testing. This revision was based on the extensive research showing a strong correlation between the development of life-threatening opportunistic illnesses and the absolute number (per microliter of blood) or percentage of CD4+ T-lymphocytes.

Table 4a.1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adults and adolescents

CD4 cell count	Clinical Category A	Clinical Category B	Clinical Category C
1. ≥500 cells/mm³	A1	B1	C1
2. 200–499 cells/mm³	A2	B2	C2
3. <200 cells/mm³	A3	B3	C3

Category A Conditions	Category B Conditions	Category C Conditions
■ No symptoms ■ Acute HIV infection (resolves) ■ Generalized lymphadenopathy	■ Bacillary angiomatosis ■ Oropharyngeal candidiasis ■ Vulvovaginal candidiasis: persistent, frequent, or poorly responsive to therapy ■ Cervical intraepithelial neoplasia II or III ■ Constitutional symptoms: fever, diarrhea >1 month ■ Oral hairy leucoplakia ■ Herpes zoster: multiple episodes or involving >1 dermatome ■ Idiopathic thrombocytopenic purpura ■ Listeriosis ■ Pelvic inflammatory disease: particularly if complicated by tubo-ovarian abscess ■ Peripheral neuropathy	■ Candidiasis of bronchi, trachea, lungs or oesophagus ■ Invasive cervical cancer ■ Coccidioidomycosis, disseminated or extrapulmonary ■ Cryptococcosis, extrapulmonary ■ Cryptosporidiosis (intestinal infection >1 month duration) ■ Cytomegalovirus disease (excluding liver, spleen or lymph nodes) ■ HIV-related encephalopathy ■ Herpes simplex: chronic ulcer >1 month duration, or bronchitis, pneumonitis or oesophagitis ■ Histoplasmosis: disseminated or extrapulmonary ■ Isosporiasis: >1 month duration ■ Kaposi's sarcoma ■ Burkitt's lymphoma ■ Immunoblastic lymphoma ■ Primary lymphoma of the brain ■ *Mycobacterium avium* complex or *M. kansasii*: disseminated or extrapulmonary ■ *M. tuberculosis*: any site ■ *Mycobacterium*: other species or unknown species, disseminated or extrapulmonary ■ *Pneumocystis carinii* pneumonia ■ Recurrent pneumonia ■ Progressive multifocal leucoencephalopathy ■ *Salmonella* septicaemia, recurrent ■ Toxoplasmosis of the brain ■ Wasting syndrome due to HIV

Table 4b.1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adults and adolescents

	Clinical categories		
CD4+ T-cell categories	(A) Asymptomatic, acute (primary) HIV or PGL	(B) Symptomatic, not (A) or (C) conditions	(C) AIDS-indicator conditions
(1) ≥500/μL	A1	B1	**C1**
(2) 200–499/μL	A2	B2	**C2**
(3) <200/μL AIDS-indicator T-cell count	**A3**	**B3**	**C3**

Note: Bold type, i.e. A3, B3 and C1-3, indicates an AIDS diagnosis.

4.3.2 WHO Clinical Staging system for HIV/AIDS[50-53]

First published in 1990 this staging system is principally based on clinical criteria. It has been revised and updated in 2007 and is chiefly used for making a decision on starting Anti – retroviral therapy in resource constrained settings with or without laboratory assessments of CD4+ T-lymphocyte levels. Clinical stages are categorized as 1 through 4, progressing from primary HIV infection to advanced HIV/AIDS.

Primary HIV Infection

- Asymptomatic
- Acute retroviral syndrome

Clinical Stage 1

- Asymptomatic
- Persistent generalized lymphadenopathy

Clinical Stage 2

- Moderate unexplained weight loss (<10% of presumed or measured body weight)
- Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis)
- Herpes zoster
- Angular cheilitis
- Recurrent oral ulceration
- Papular pruritic eruptions
- Seborrheic dermatitis
- Fungal nail infections

Clinical Stage 3

- Unexplained severe weight loss (>10% of presumed or measured body weight)
- Unexplained chronic diarrhea for >1 month
- Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant)
- Persistent oral candidiasis (thrush)
- Oral hairy leukoplakia
- Pulmonary tuberculosis (current)
- Severe presumed bacterial infections (eg, pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)
- Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
- Unexplained anemia (hemoglobin <8 g/dL)
- Neutropenia (neutrophils <500 cells/µL)
- Chronic thrombocytopenia (platelets <50,000 cells/µL)

Clinical Stage 4

- HIV wasting syndrome, as defined by the CDC (see Table 3, above)
- Pneumocystis pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or
- visceral herpes at any site)
- Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)
- Extrapulmonary tuberculosis
- Kaposi sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Cryptococcosis, extrapulmonary (including meningitis)
- Disseminated nontuberculosis Mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Candida of the trachea, bronchi, or lungs
- Chronic cryptosporidiosis (with diarrhea)
- Chronic isosporiasis
- Disseminated mycosis (eg, histoplasmosis, coccidioidomycosis, penicilliosis)
- Recurrent nontyphoidal Salmonella bacteremia
- Lymphoma (cerebral or B-cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy
- Symptomatic HIV-associated cardiomyopathy
- Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

5. Laboratory Diagnosis

It is a well-established fact that 20-80% of the people in different parts of the world who have HIV infection do not know their HIV status.[55]Hence, every opportunity is offered to these people to make use of the tests available to determine their HIV status. Since HIV is acquired most frequently through unprotected sexual contact, a number of moral, ethical, legal and psychological issues are related to HIV testing. Nevertheless, laboratory tests are the only means in establishing the diagnosis of HIV.

5.1 Who to Offer HIV test?

- Individuals with clinical features suggestive of HIV infection,
- Patients with tuberculosis, especially the young,
- Patients having sexually transmitted illnesses (STIs),
- Antenatal care patients,
- Patients with hepatitis B and C co-infection,
- History of high-risk behavior/ transfusion,
- Patients undergoing invasive surgical procedures.[55-57]

5.2 Purpose of HIV Testing[58,59]

- To identify asymptomatic and symptomatic HIV positive individuals
- To diagnose clinically suspected cases
- To plan prophylaxis, medical management, and treatment of HIV and related illnesses
- To plan personal and family's future if the result is positive
- To assure safety of blood and blood – related products
- To motivate for behavior changes through counseling among those high – risk behavior individuals who tested anti – HIV negative
- To induce behavior change and prevent further HIV transmission by counseling in those individuals who tested anti – HIV positive
- To monitor trends of HIV epidemic

5.3 HIV Serology

Vast majority of people have detectable antibodies to HIV by the end of three months following HIV infection. These can be detected by standard enzyme linked immunoassays (ELISA), the first HIV screening tool. However, during the window period i.e. the time taken by an individual with HIV infection to react to the virus by producing HIV antibodies, ELISA tests may be false negative. The window period is usually between 3 to 6 weeks (on average 22 days) following infection. During this period the infected individual is highly infectious but anti-HIV seronegative. Antigen testing shortens this window period to approximately 16 days and nucleic acid amplification testing (NAAT) further reduces this period to 12 days.[60]

Table 5.Laboratory Tests employed for HIV diagnosis[61-63]

- Screening tests
  - ELISA
  - Rapid tests

- Supplemental or confirmatory tests
  - Immunofluorescent assay (IFA)
  - Western blot
  - Lineimmuno assay (LIA)
  - Radioimmuno precipitation assay (RIPA)

- Others
  - p24 antigen
  - Culture
  - Polymerase chain reaction (PCR)
  - Plasma/ serum viral load

- Alternative to classical tests
  - Oral fluid (saliva) HIV tests
  - Urine tests

5.3.1 ELISA

It is the most widely used screening tool due to high sensitivity and ability to test large number of samples. Currently there are four generations of ELISA tests available to diagnose HIV.

I generation – whole viral lysates

II generation – recombinant antigen

III generation – synthetic peptide

IV generation – antigen + antibody i.e. simultaneous detection of HIV antigen and antibody[64]

The principles of ELISA are classified as indirect, competitive, sandwich, and capture essay. As per National HIV testing policy, two ELISA using different principles are required to diagnose HIV infection (in a clinical setting).[64]

5.3.2 Rapid Tests

These tests yield result in less than 30 min and can be read by naked eye. Hence, they have found applications in emergency room, physician's office, autopsy room, smaller blood banks and resource limited, remote or field settings. Different types are available like dot blot assays (tridot), particle agglutination, HIV spot and comb tests, and fluorimetric microparticle technologies.

5.3.3 Western Blot

It is a more specific assay where antibodies against numerous proteins are detected. If the sample has antibodies, colored bands appear wherever human IgG binds to the viral proteins on the strip. The WB test can be negative, positive or indeterminate. If no viral bands are detected, the result is negative. If one viral band for each of the groups gag, pol, and env gene product is present then the result is positive. In circumstances when few viral bands are detected not enough to confirm diagnosis, the result is considered indeterminate and the individual is retested at a later date. There are several criteria's to interpret the test results but the WHO criterion is commonly employed.

Table 6. Positive Western Blot Criteria[64]

Organization	Criteria
WHO	2 env with/ without gag/pol
CDC	Any two – p24, gp41, gp120/160
DuPont	p24 and p31, and gp41 or gp120/160

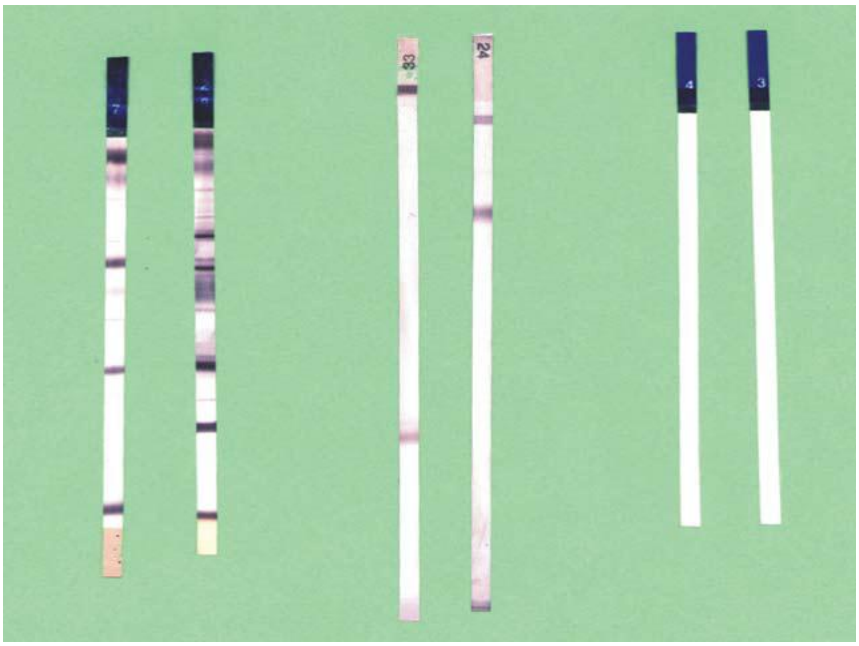

Fig.7. Examples of HIV-1 WB results
(Left two membranes: anti HIV-1 positive samples; middle membranes: anti-HIV-1 indeterminate samples; right two membranes: anti-HIV-1 negative samples)

5.3.4 p24 Antigen Test

This test helps diagnose early HIV infection when antibodies are not detected in the blood. Levels of p24 antigen increase significantly between one to three weeks after initial infection. It is during this time frame the test finds its application. Also it is used to detect HIV infection in new born when presence of maternal antibodies interfere with routine screening procedures. The major drawback of this test is its low sensitivity which may be due to the reason that free p24 antigen may complex with p24 antibody.

5.3.5 PCR[64,65]

Here the target HIV RNA or the proviral DNA is amplified in vitro by chemical reaction thus making it an extremely sensitive assay. Three different techniques namely RT – PCR, nucleic acid sequence based amplification (NASBA) and branched – DNA (b – DNA) assay have been employed in establishing diagnosis of HIV.

With "ultrasensitive" protocols developed for all three methods the detection limit of 20 – 50 HIV-1 RNA copies/ml has been reached. The HIV-1 viral load usually ranges between 102 and 107 HIV RNA copies/ml in untreated individuals. Persistently detectable viremia and high baseline levels are predictors of poor prognosis, while risk of progression of HIV infection to AIDS is low if viral titers are <10,000 HIV RNA copies/ml.

5.4 Isolation of HIV

HIV can be isolated from peripheral blood mononuclear cells or plasma and other body fluids. Majority of cultures in untreated HIV positive patients are usually positive in 2 weeks.

However isolation of virus is a time consuming expensive procedure demanding expertise, and proper containment facilities. It is currently used for research purposes only.

Basic assays for screening include ELISA and Rapid assays. Western blot (WB) is a confirmatory tests for HIV antibody. WB confirmation is expensive, time-consuming, technically complex and lacks standardization both in method and interpretation, resulting in assays with variable

specificity and sensitivity.[66] A study conducted at Swedish Institute for Infectious Disease Control showed that a combination of specific and sensitive anti-H1V ELISAs based on different test principles and different antigen sources could be used in parallel or sequentially either as a complement or an alternative to WB for verification of the HIV antibody status.[66] If a combination of ELISAs is used as an alternative to WB, sera reactive on any ELISA should be repeatedly analyzed by the two ELISAs or tested by a third screening assay.[66]

6. CD4 T Lymphocytes in HIV

CD4 T Lymphocytes are also known as CD4 T cells or, T-helper cells. CD4 T Lymphocytes has been identified as the major receptor for HIV fusion and entry into the cell. CD4 T Lymphocyte dysfunction, both quantitative and qualitative is the hall mark of HIV infection, thus serving as surrogate marker for monitoring disease progression.

6.1 Effects of HIV on CD4 T Lymphocytes

Table 7. Effects of HIV on CD4 T-Cells[67]

Direct Effect	Indirect Effects
Infection and resultant cytotoxicity with loss of absolute numbers	Decreased CD4 + T cell proliferation and differentiation Dysregulation and decreased production of IL-2 and other cytokines Decreased IL-2 receptor expression Defective T cell colony formation

6.2 Mechanism of CD4 T Lymphocyte Depletion in HIV

Table 8. Mechanism of CD4 T-cell Depletion in HIV[67]

Direct	Indirect
Accumulation of unintegrated viral DNA Interference with cellular RNA processing Intracellular auto fusion of gp 120-CD4 Loss of plasma membrane integrity because of viral budding Elimination of HIV infected cells by virus specific immune responses	Aberrant intracellular signaling events Syncitium formation Auto immunity Super antigenic stimulation Innocent bystander killing of viral antigen coated cells Apoptosis Inhibition of lymphopoiesis

6.3 CD4 T Lymphocyte Estimation

CD4 cell count estimation is done to evaluate the strength of the immune system of an individual diagnosed to have HIV. Over a period of time the CD4 count witnesses a gradual drop in its level following HIV infection. Fall below a critical level increases the likelihood of opportunistic infections in the individual.

CD4 count estimation helps in some of the key decisions towards management of HIV positive patients. The counts can help determine;

- The risk of developing opportunistic infections and subsequently to decide prophylactic treatment against opportunistic infections
- Stage of HIV infection and also to initiate ART
- Response to ART

CD4 T cell count estimated at the time of diagnosis of HIV helps forming a baseline value for future monitoring of disease progression. It is repeated every 3-6 months depending on the health status of the individual. The pattern of CD4 + cell counts over time is more important than any single CD4 + value. Normal CD4 cell count is > 500 cells/μL. CD4 counts normally undergo diurnal variation with fluctuations of as much as 150-300 cells/mm3 difference between morning and evening values in normal hosts.[68]

CD4 T cell estimation is done using flow cytometry, which was developed with the aim to assess cloning efficiency, growth rate and phenotype of T cell clones. The simultaneous phenotyping allows precursor cell analysis under conditions that stimulate growth of more than one cell type.

Francis. E. Mandy et al. described a method for simultaneous analysis of CD3, CD4 and CD8 positive cells from whole blood utilizing single laser flow cytometers.[69]All three T values are attained from a single test tube. CD4 and CD8 positive cells are identified only if they are CD3 positive. Thus the values obtained by this method for T helper/inducer and T cytotoxic/suppressor cells can be reported directly as a percentage of T-lymphocytes.

Even though the gold standard method for detection of CD4 + T lymphocytes count is flow cytometry, the determinations of these cell counts is not easily obtained in many settings often for financial or logistical reasons. Several alternatives to flow cytometry analysis of CD4 + cell counts have been developed.

7. HIV In Developing Countries - Challenges

The epidemic of HIV is not uniformly spread across the world. Over 90% of HIV infected people live in developing world.[70] According to UNAIDS epidemic update 2009, Sub-Saharan Africa is the worst hit, being home to about 67% of HIV infections worldwide, 68% of new HIV infections among adults and 97% of new HIV infections among children.[71] This region accounted for 72% of AIDS related death in 2008.[71] Asia, home to 60% of world's population, stands second only to Sub-Saharan Africa in terms of number of people living with HIV.71 India roughly accounts for half of Asia's HIV prevalence.[71]

There are multitude of factors involved in the diverse geographic variation and high prevalence of HIV in developing countries. Political factors, poverty, illiteracy, social stigmas, religious stigmas, presence of other infections favoring transmission of HIV,[72] ethical issues concerning testing and treatment and several other complex and diverse factors have been responsible for high HIV prevalence in the developing regions. In the developing world heterosexual mode of transmission of HIV remains a dominant mode of HIV transmission and presence of STI's in sexually active individuals increases the transmission rate several fold. Mother-to-child transmission has been on the rise in the developing countries.

Lack of individual's awareness for voluntary HIV testing and counseling due to various pervasive social and religious stigmas is one of the barriers for a successful HIV surveillance program in developing countries. Further, limitation of resources for HIV testing and monitoring and inadequate facilities for social support and treatment of those tested positive have been the known barriers for HIV containment.

Laboratory support is critical for diagnosis of HIV infection. Diagnosis of HIV requires serological testing and CD4 T lymphocyte count is prerequisite for initiation of ART (Anti-Retro Viral Therapy). Laboratory support is crucial for the success of AIDS programs, the infrastructure, expertise and networking require strengthening in many developing countries. In the era necessitating widespread use of investigations to diagnose and monitor the treatment of HIV infection and its response to HAART therapy respectively, imposes financial and technological constraints towards upgrading laboratory facilities. For instance, single CD4 count estimation by flow cytometry is approximately$ 30(US), and the complete cost on the basis of quarterly monitoring amounts to $120(US) per year per person.[73]Because resources are currently so constrained in developing countries (both financial and technological), it is imperative that available funds be spent for interventions that are cost-effective. This has led to the resurgence of interest in low cost surrogate markers for monitoring the disease activity, progression and it's response to HAART in HIV infected individuals, living in resource-limited environments most heavily impacted by the epidemic.

8. Surrogate Markers[74-76]

Surrogate markers of HIV infection are measurable traits that correlate with the development of clinical AIDS. Ideally, such markers should;

1) Allow patients at highest risk of disease progression to be identified.

2) Aid in estimating the duration of infection.

3) Assist in disease staging.

4) Predict development of indicator disease (opportunistic infections of AIDS).

5) Measure, in vitro, the therapeutic efficacy of HAART

6) Must be easily quantifiable, reliable, clinically available, and affordable.

A long list of such markers has been identified as suitable surrogate markers of HIV infection. CD4 T-lymphocyte, Total lymphocyte count, Hemoglobin, Hematocrit, β2-microglobulin, Neopterin, Soluble Interleukin-2 receptor, Viral load estimation and many others.

9. CD4 T-Lymphocyte Count vs. Total Lymphocyte Count

WHO guidelines recommend CD4 count testing to be performed to monitor the HIV infected patients.[77] But given the financial constraints in developing countries like India, WHO recommended use of Total Lymphocyte Count (TLyC) to monitor the Immune response to HAART. TLyC has already been a useful tool in low-income countries for predicting immunosuppression and triggering opportunistic infection prophylaxis. A study done in Ahvaz, a city in South of Iran showed that TLyC to be a suitable predictor of CD4 count with 75% sensitivity.[78] Another Study in China found that for the 131 HIV-infected patients without ART, TLyC >/= 1076 cells/mm3 was the best point to indicate CD4 of >/= 200 cells/mm3,

with a 78.12% sensitivity and a 74.75% specificity.[79] Some studies have shown that TLyC provides easy and accurate measure to monitor progression to AIDS in HIV-infected persons in resource poor settings.[80] However, recent studies have suggested that it is not a useful tool in monitoring Anti –Retroviral Therapy (ART) but in situations where CD4 testing is not available TLyC can be used for decision-making.[81]

This study would help correlate the changes in TLyC as against CD4 count with respect to clinical profile of newly diagnosed HIV positive patients and determine its value as a low cost alternative surrogate marker to CD4 cell count in HIV positive patients.

MATERIALS & METHODS

1. **Study Design:**

   A Prospective Study

2. **Source of Data:**

   50 patients who were tested positive for HIV by appropriate testing methods as per CDC surveillance guidelines and National norms were selected for the study. The study was conducted at Sri. R.L. Jalappa Hospital and Research Centre, Tamaka, Kolar.

3. **Inclusion Criteria:**

   Patients of both sexes aged >18 years who are admitted with HIV positive infection for the first time at above mentioned hospital

4. **Exclusion Criteria:**

   Patients <18 years of age

   Diagnosed cases of HIV positive and/or those already on treatment for the same

5. **Method of Collection of Data:**

   50 consecutive cases of HIV positive satisfying the inclusion-exclusion criteria will be selected for the study. Once patient wass selected, a careful history was recorded followed by general physical and systemic examination. Routine investigations were done which included Rapid spot test, ELISA, CD4 cell count, complete hemogram with peripheral smear, urine routine and microscopy, stool examination, chest X-ray, blood sugar,

blood urea, serum creatinine and others. Special investigations like ultrasonography (USG) of abdomen, CT, MRI, serous fluid analysis etc., as the clinical condition warrants.

Diagnosis of HIV was made in these patients by detailed clinical history, physical examination, Routine laboratory investigations (2 ELISA or 1 ELISA & 1 Western Blot). Venous samples were sent for evaluation of CD4 count (by Flow cytometry method). At the same time samples were sent for evaluation of TLyC.
Total lymphocyte counts TLyC was calculated by multiplying the differential lymphocyte count with the total leucocyte count.

TLyC=Total leucocyte count (TLC) x Differential lymphocyte count]

Collection and processing of specimens

Various samples e.g. sputum, oral swab, blood, stool, urine, cerebrospinalfluid (CSF), lymph node aspirate were collected as per symptoms and clinicalpresentations. All the specimens were collected under universal asepticprecautions in suitable sterile containers.

Oral swab

Oral swabs were collected in cases presenting with oral thrush. Two sterile swabs were taken and each rubbed against the right tonsil and rolled along the softpalate to the left tonsil. Curdy white patches were also swabbed. Gram staining was done with one swab and the other swab was streaked on sabourauds dextroseagar slope. Germ tube test was also performed for presumptive identification of Candida albicans

Stool

Fresh samples of stool were collected in sterile, dry, leak proof, wide-mouthcontainer.Wet mount- Direct saline mounts and iodine mounts were screened undermicroscope for helminthes eggs, larvae, protozoan cysts, trophozoites, pus cellsand possible fungal elements. After concentration method supernatants were discarded, sediment was used for Kinyoun cold test was done for detection ofoocysts of cryptosporidium, cyclospora, isospora and sarcocystis.

Sputum

Early morning sputum was collected in a wide mouthed sterile container.Instructions were given to rinse the mouth with tap water before samplecollection, and to collect expectoration and not saliva.

Wet mount- sputum sample was taken on a clean glass slide and 2 drops of 10%KOH was added and covered with a cover slip. Slip was kept in incubator at 37℃ for 10mins and examined for fungal elements.

Staining of sputum was done for detection of

- Gram stain- to see pyogenic bacteria pus cells and epithelial cells
- Ziehl- Neelsen stain for acid fast bacilli
- Methanamine silver nitrate stain- for the detection of pneumocystis jerovecii cysts

Urine

Mild stream urine samples were collected in sterile container. All the samples were processed within one hour of collection and Gram stain done to detect pus cells, epithelial cells, bacteria and yeast cells.

Cerebrospinal fluid (CSF)

CSF samples were obtained by lumbar puncture and processed immediately

CSF fluid was sent to microbiology for

- Culture
- Staining- Gram stain, Ziehl-Neelsen's stain and methylene blue stains were used to detect different pathogens
- India ink wet mount- it was done to detect presence of Cryptococus.

Blood

Skin at the site of venepuncture was cleaned with 70% alcohol followed by2% tincture iodine. Total 15ml blood was collected from each patient. 10ml was used for blood culture and 5ml was collected in sterile pencillin vial for serological tests.

Serology

The blood collected in pencillin vial was allowed to clot. Samples were centrifuged and serum was collected for following tests

- VDRL
- HbsAg
- HCV

6. Statistical Methods:

Data analysis was done using SPSS for Windows version 16.0. Mean was used as measures of central tendency and Standard Deviation was used as measure of dispersion for descriptive statistics. Paired and unpaired Student t-test with one tailed and two tailed significance was used to analyze the difference between the means.

Using the above statistical measures correlation was analyzed between TLyC and CD4 cell count. Further attempts were made to derive any statistically significant correlations between CD4 cell count and other parameters like Hb%.

RESULTS

A total of 50 HIV positive patients who were admitted at Sri R. L. Jalappa Hospital & Research Centre, Tamaka, Kolar, who met the inclusion and exclusion criteria were clinically examined and subjected for relevant laboratory investigations. The data obtained was tabulated and analyzed.

The following observations were made.

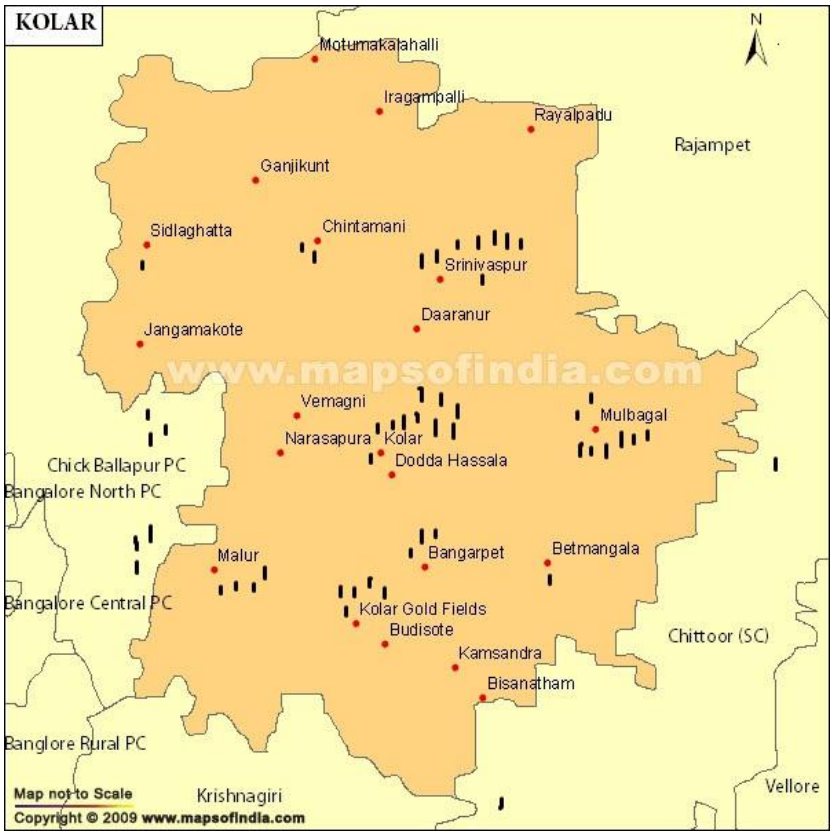

Fig.8: Region wise distribution of cases

The above map of Kolar district shows the region distribution of the patients included in the study. Majority of the patients admitted in the hospital were from the Kolar city, Mulbagal, Srinivaspur, Kolar Gold Field, Bangarpet and Malur areas of Kolar district.

1. Sex & Age Distribution

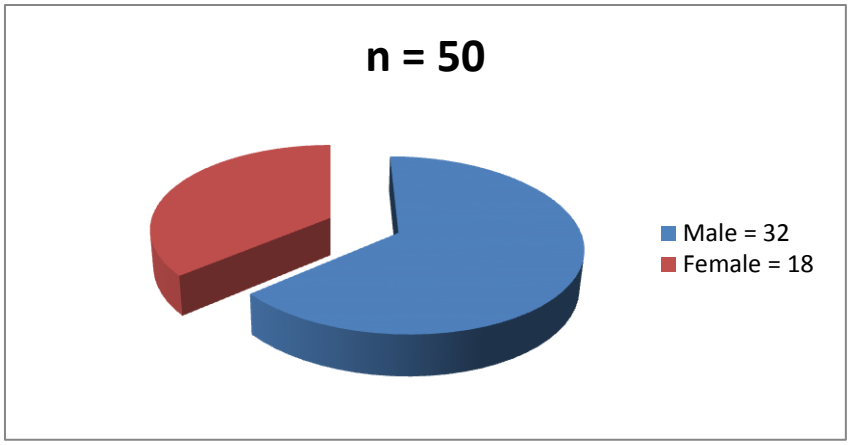

Fig.9: Pie Chart: Sex Distribution of Cases

Table 9: Age & Sex Distribution

Age	Male	Female	Total
20-29	8	5	13
30-39	10	5	15
40-49	9	5	14
50-59	4	2	6
>60	1	1	2
Total	32	18	50

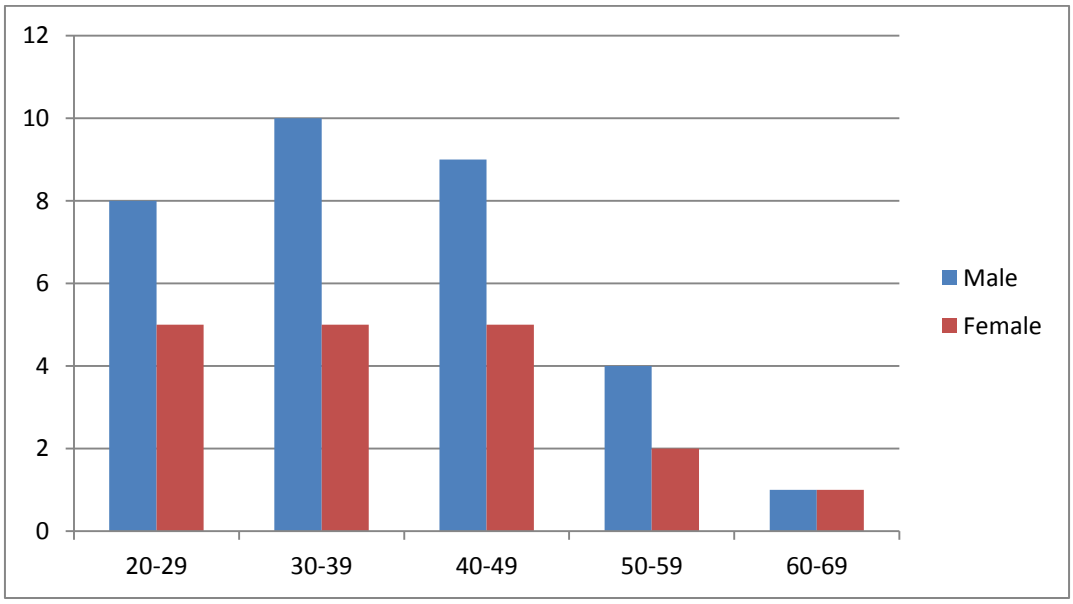

Fig.10: Age & Sex Distribution

Observations:

Of the 50 patients included in this study 32 were males i.e. 64% and the rest females with male to female ratio of 1.78: 1. A total of 42 patients in the study belonged to the age group 20 to 49 years comprising 84% of study subjects. Males had the higher incidence of 64.29% in the age group 20 to 49 years.

1.1 Sex & Age: Correlation with TLyC and CD4 Count

Table 10: Age: TLyC and CD4 correlation – TOTAL Sample

Age	N = 50	Mean TLyC	Mean CD4 count	r Age & CD4 Count	r TLyC & CD4 Count
20-29	13	2041.61	258.88	0.047	0.278
30-39	15	1697.07	406.97	0.027	-0.129
40-49	14	1885.57	265.02	0.047	0.480
50-59	6	1608.25	374.44	-0.323	0.165
>60	2	1109	220.02	1.000*	1.000*
Mean		1668.3	305.07		

Table 11: Age & Sex: Correlation between TLyC & CD4 – MALE Subjects

Age	N = 32	Mean TLyC	Mean CD4 count	r Age & CD4 Count	r TLyC & CD4 Count
20-29	8	2119.88	302.56	-0.110	0.104
30-39	10	1707.33	310.27	0.165	-0.462
40-49	9	1507.33	164.99	-0.493	0.246
50-59	4	2161.5	143	-0.239	0.993*
>60	1	268	34.74	**	**
Mean		1552.81	191.11		

Table 12: Age & Sex: Correlation between TLyC & CD4 – FEMALE Subjects

Age	N = 18	Mean TLyC	Mean CD4 count	r Age & CD4 Count	r TLyC & CD4 Count
20-29	5	1963.34	215.19	0.139	0.828
30-39	5	1686.8	503.66	0.131	0.366
40-49	5	2263.8	365.04	0.962*	0.732
50-59	2	1055	605.88	-1.000*	1.000*
>60	1	1950	405.29	**	**
Mean		1783.79	419.01		

* Correlation is significant at the 0.01 level (2-tailed).
** Cannot be computed because at least one of the variables is constant.

Group and sub group analysis was done for all the 50 subjects to look for significant correlations between different age groups with CD4 cell count and between TLyC and CD4 cell count for the respective age groups. Descriptive analysis was also carried out to compute mean TLyC and CD4 count for different groups and sub groups.

The Mean TLyC and CD4 Cell count for male subjects is 1552.81 cells/µL and 191.11 cells/µL respectively. Male patients aged 30 years and above had mean CD4 cell count less than 200 cells/µL. The correlation between age and CD4 cell count showed no significant correlation between any of the age groups. Likewise, no significant correlation was observed between TLyC and CD4 cell count of the corresponding age groups in male subjects, except for age group 50-59 years, but smaller sample size makes it difficult to validate this correlation.

The female subjects had higher Mean TLyC and CD4 cell counts as compared to male subjects. The mean TLyC and CD4 cell counts were 1783.79 cells/µL and 419.01 cells/µL respectively. The correlation between age and CD4 cell count for female subjects showed significant correlation for 7 subjects between age group

40-59 years. Again small sample size was observed in this group. Correlation between TLyC and CD4 cell counts was again not significant for corresponding age groups among female subjects.

Overall, age did not have a significant effect on correlation with CD4 cell count. The correlation between TLyC and CD4 cell count for respective age groups was also not significant.

2. Marital Status

Table 13: Marital Status of Subjects

Marital Status	Married	Single	Divorced/ Spouse Expired
Male	29	2	1
Female	18		

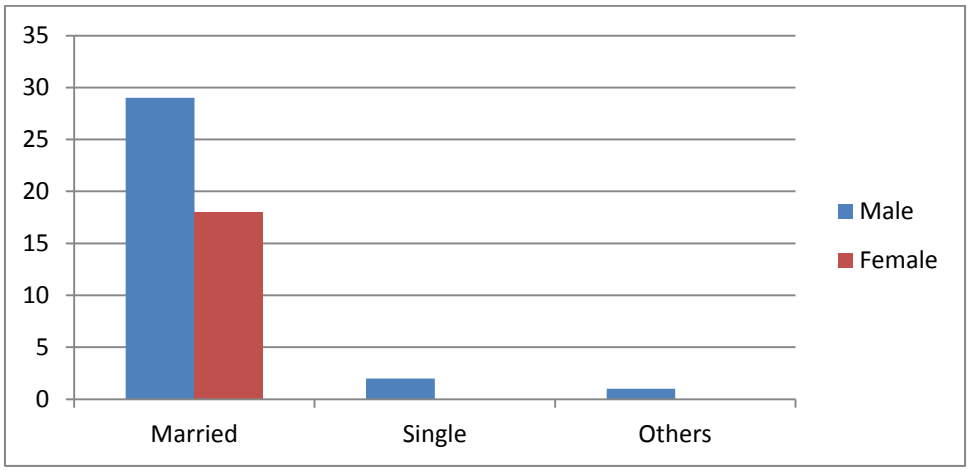

Fig. 11: Marital Status of Subjects

48 patients in the study were married with 1 male patient having the history of spouse expired in the last 6 months who was tested positive for HIV before death.

3. Risk Factors

28 of the 32 male subjects i.e. 87.5% in the study had given the history of having unsafe sexual practices with a partner(s) other than his spouse. This high risk sexual behavior was found to be a strong factor involved in HIV transmission among the sexually active adults. On the contrary, all the 18 female subjects i.e. 100% had contracted HIV from their spouses, who were tested positive either before or at the time of diagnosing the female subjects. Some of the spouses of HIV positive females were on ART, but gave history of not resorting to safe sexual practices resulting in spreading the virus. Females in this study were innocent victims of this viral transmission from unsafe sexual practices of their spouses.

No report of viral transmission through blood and blood components, contaminated needles, injection drug abuse or vertical transmission was observed in the study.

Another commonly observed behavior among the male subjects of the study was smoking and alcoholism. 65.66% of the male subjects had history of either cigarette smoking or alcohol consumption and 25% of patients had both the risk factors. There was no male subject in the study with absence of both the risk factors.

Table 14: Smoking & Alcoholism In Male Subjects

Smoking	21	65.66%
Alcohol	21	65.66%
Smoking & Alcohol	8	25%
None	-	

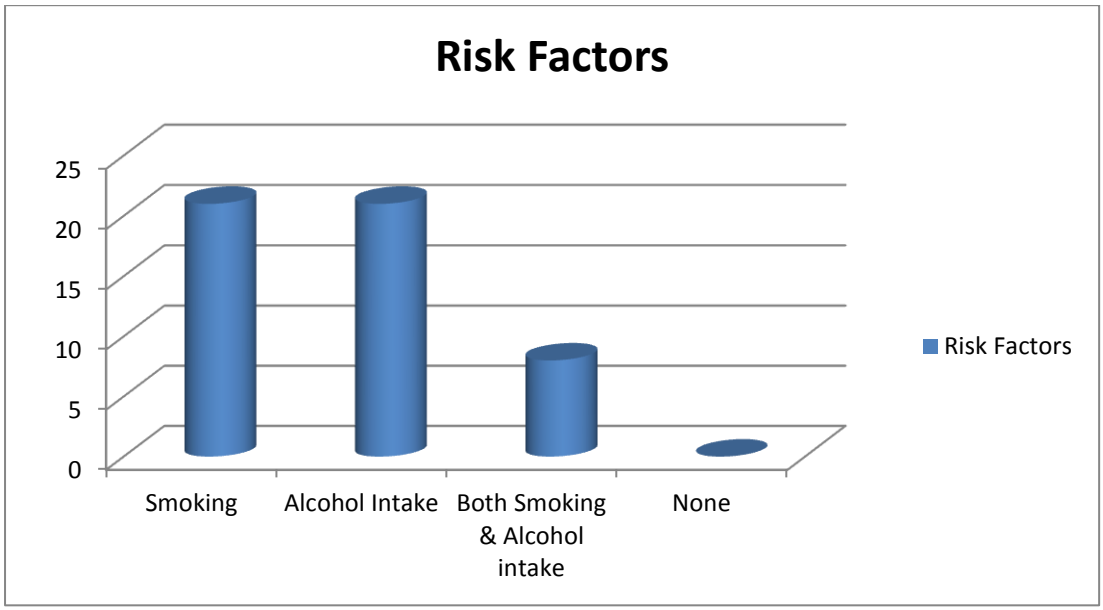

Fig.12: Smoking and Alcohol Prevalence in Male Subjects

Occupation data was tabulated to study prevalence of HIV in different occupations and to identify high risk occupations based on high prevalence of HIV.

Table 15: Occupation of the Study Subjects

Sl.Number	Occupation	Male	Female	Total
1	Transport	9		9
2	Farmer	5	1	6
3	Manual Labor	9	1	10
4	Homemaker		15	15
5	Cop	4		4
6	Others	5	1	6

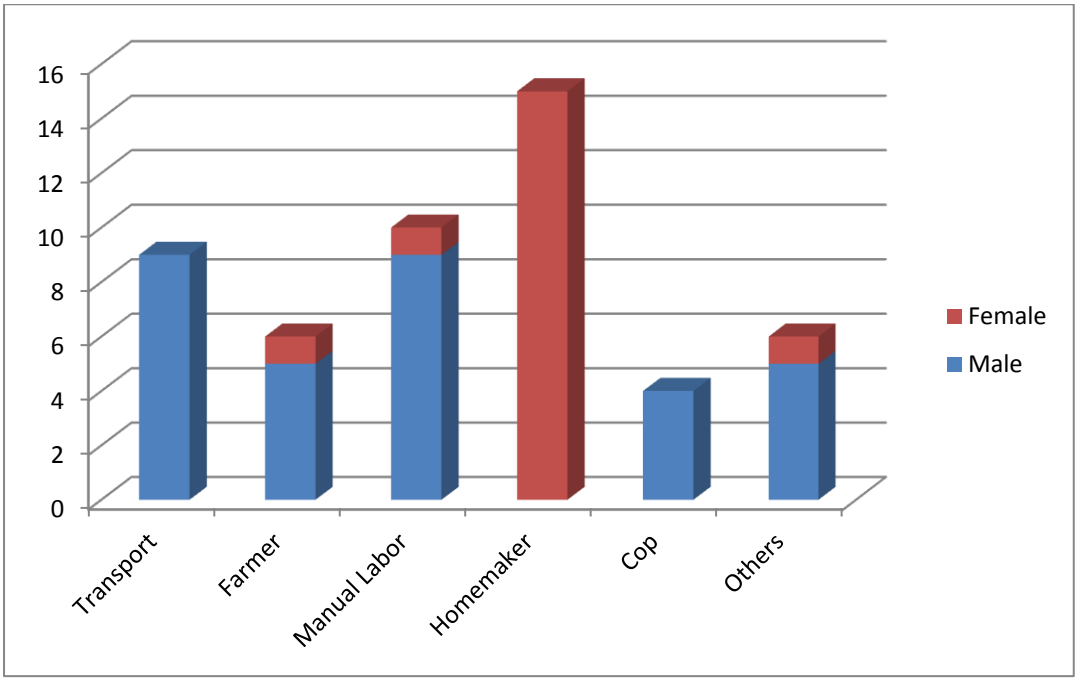

Fig.13: Occupation of Study Subjects

Of the 50 cases studied 9 patients were working in the transport department i.e 18%, and all of them were male subjects. 10 patients (20%) were manual labors, 6 (12%) were farmers and 15 female subjects were homemakers i.e. 30%. Others were miscellaneously categorized as cops, teacher, businessmen etc.

Amongst the male subjects those working in transport department and as manual labors had the highest incidence of HIV, 28.13% each.

4. Presenting Symptoms

The patients with HIV presented with various specific and no specific symptoms at the time of admission. These symptoms were tabulated to understand the frequency of common presenting complaints among the HIV patients.

Table 16: Common Presenting Symptoms

Symptom	Total Number of Patients
Fever	19
Abdominal Pain	4
Nausea & Vomiting	13
Loose Stools	7
Cough with or without Expectoration	13
Constitutional Symptoms	5
Altered Sensorium	5
Skin Rash	2
Genitourinary Complaints	3
Routine Screening	14

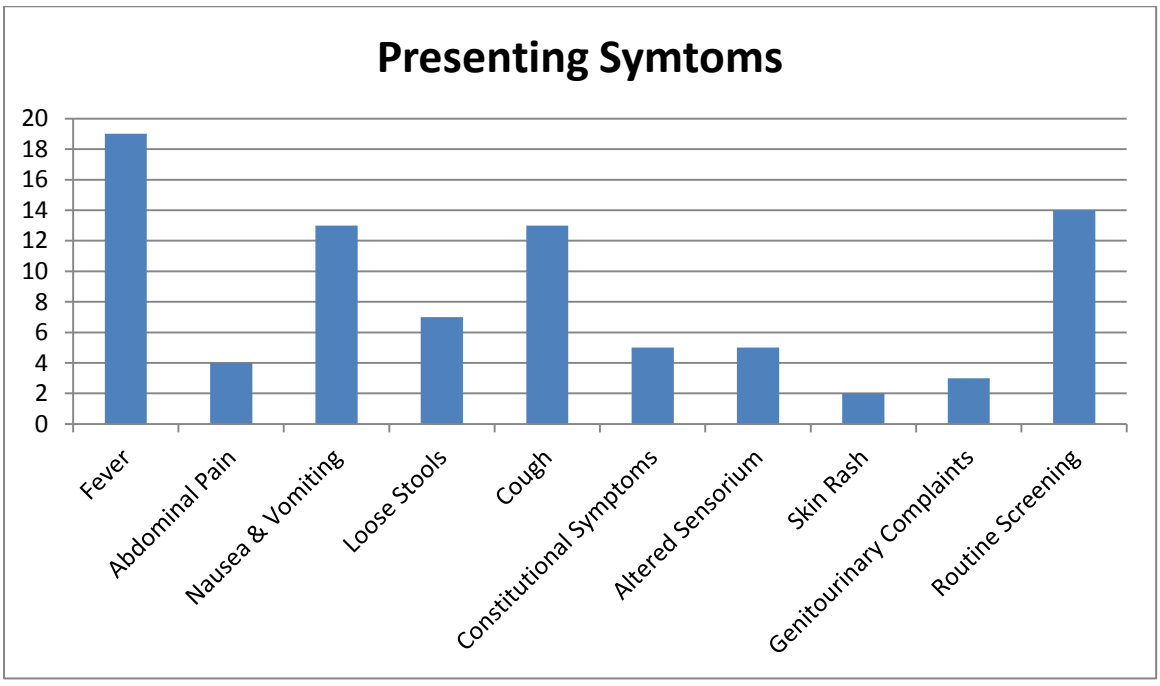

Fig.14: Common presenting Symptoms

Out of the 50 patients studied, fever was the most common presenting symptom. 19 patients presented with fever i.e. 38%. Other common symptoms were gastro intestinal symptoms like abdominal pain in 4 patients (8%), nausea and vomiting in 13 patients (26%), loose stools in 7 patients (14%). Cough with or without expectoration and breathlessness was the most common respiratory complaint and 13 patients (26%) presented with this complaint. Diagnosis of pulmonary tuberculosis (clinical and radiological with or without sputum positivity) was made in 4 subjects (8%) of which 2 were known cases and defaulters. Constitutional symptoms like generalized weakness, decreased appetite, weight loss, fatigability and lethargy was present in 5 patients (10%). 5 patients i.e. 10% presented with central nervous system (CNS) complaint of altered sensorium (disorientation) with 2 patients having an episode of seizure before admission. Diagnosis of herpes zoster and oral candidiasis was made in one subject each at the time of admission. 3 patients had genitourinary complaints of inguino-scrotal swelling and penile ulcer at the time of admission.

13 patients (26%) were admitted under obstetric and surgical wards for other complaints that on routine screening tested positive for HIV. 1 patient who was admitted in medical emergency following OP compound consumption also tested positive for HIV on routine screening.

5. Clinical Signs

Clinically, examination of the 50 patients showed fever, pallor, lymphadenopathy and skin and mucosal changes to be the most common findings present in many cases.

A summary of the common clinical signs brought out on examination is shown in the table and figure below;

Table 17: Most Common Clinical Signs

Clinical Sign	Number of Subjects
Fever	20
Pallor	19
Skin Changes	26
Lymphadenopathy	10

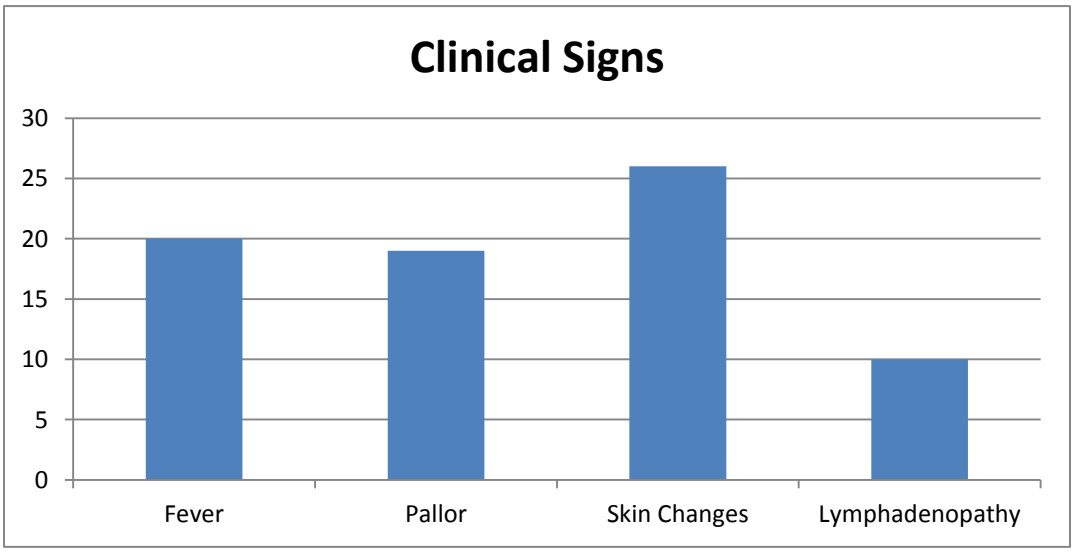

Fig.15: Most Common Clinical Signs

20 patients in the study had fever as one of the presenting complaints which was confirmed further on clinical examination. The fever was present either as a part of a systemic illness or occurred along with constitutional symptoms as a non-specific illness.

Examination further showed 19 patients having pallor clinically which were further confirmed with laboratory investigation like Hb estimation and peripheral smear examination. Majority (84.21%) of the patients had normocytic normochromic anemia with or without thrombocytopenia.

Most common skin change that was observed clinically was xerosis of skin observed in 24 of 26 patients. The other 2 patients had Herpes zoster and mucocutaneous candidiasis.

Lymph node enlargement was seen in 10 patients with 90% having features of reactive lymphadenitis and 1 patient having tubercular lymphadenitis on Fine Needle Aspiration Cytology (FNAC).

An attempt was made to study the mean Hb, TLC, TLyC and CD4 count in these patients and to determine the status of Pearson correlation between Hb and CD4 count and TLyC and CD4 count. The results were tabulated and relevant graphs were drawn to understand the analyzed data.

Table 18: Clinical Signs – Correlation Between TLyC & CD4 cell count

Clinical Features	Mean Hb%	Mean TLC	Mean TLyC	Mean CD4 cell count	r Hb & CD4	r TLyC & CD4
Fever	10.73	9157.90	1478.6	263.61	-0.115	0.095
Pallor	9.28	8591.00	1509	230.66	-0.224	0.070
Skin Changes	11.27	9482.98	1762.73	308.91	0.057	0.255
Lymphadenopathy Present	10.27	6606.90	1396.2	145.21	-0.70	-0.029
Lymphadenopathy Absent	12.30	10043.00	1904.5	335.24	0.099	0.115

Significant observations made in the above analysis;

- Patients with fever had mean Hb of 10.73 g/dL. The mean TLC and TLyC were 9157.90 cells/cumm and 1478.6 cells/cumm respectively. The mean CD4 cell count was 263.61 cells/cumm. The computed r value for Hb and CD4 cell count (-0.115) and TLyC and CD4 cell count (0.095) were suggesting an insignificant correlation.
- Mean Hb of patients with pallor was 9.28 g/dL. Although mean TLyC and mean CD4 cell count were 1509 cells/cumm and 230.66 cells/cumm no significant correlation existed between Hb and CD4 cell count and, TLyC and CD4 cell count.
- Similarly, no significant p value was obtained from computation of data for patients with skin changes. The Pearson correlation for Hb and CD4 cell count and, TLyC and CD4 cell count were 0.057 and 0.255 respectively.

- Data analysis for the subjects with lymphadenopathy and without lymphadenopathy showed that the mean Hb, mean TLC, mean TLyC and mean CD4 cell count were comparatively lower for the subjects with lymphadenopathy. However, computed p value for either group between Hb and CD4 cell count were not significant. However, TLyC was suitable marker of immunosuppression to CD4 cell count in patients with lymphadenopathy ($r = -0.029$).

6. Organ System Involved

Further analysis was done to assess the most common organ system that was involved at the time of initial evaluation at admission and final diagnosis made. These were categorized as Respiratory system, abdomen, CNS, genitourinary, dermatologic, and others.

Table 19: Systems Involved

System Involved	Number of Subjects
Respiratory System	14
Gastrointestinal System	20
Neurologic	7
Dermatologic	5
Genitourinary System	3
Others	11

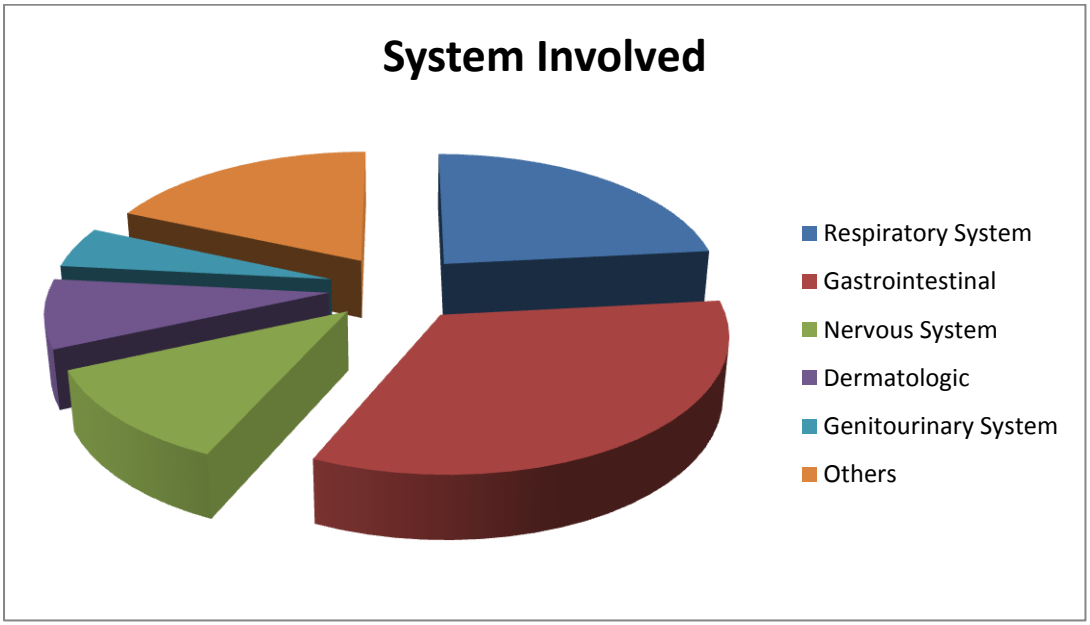

Fig.16: Systems Involved

Gastrointestinal system was the most commonly involved organ system in the study patients. 20 patients (40%) reported enteric manifestations at the time of presentation with pain abdomen, nausea, vomiting and loose stools. Gastroenteritis was the most common diagnosis made among these patients i.e. 14 patients (70% of all GI system cases). These manifestations occurred either alone or with other organ system involvement. 5 cases were reported to have acid peptic disease and 1 case of pancreatitis was diagnosed.

14 cases (28%) of respiratory illnesses were reported in this study. Pneumonia was the most common diagnosis made based on clinical and radiological findings. Of the 10 cases of pneumonia (i.e. 20% of total study subjects) 2 patients were confirmed to have Pneumocystis jirovecii pneumonia. Pulmonary tuberculosis was present in rest 4 patients of whom 2 were known cases and defaulters of anti-tubercular treatment.

7 (14%) of the study subjects presented with altered sensorium and among these 2 (28.56%) were confirmed to have Cryptococcal meningitis, 2 (28.56%) others had tubercular meningitis and 1 patient was found to have Neurocysticercosis (radiological diagnosis). Diagnosis was unconfirmed in rest 2 subjects.

10% of the patients had dermatologic complaints with 2 patients diagnosed to have herpes zoster. Oral candidiasis, cellulitis of the lower limb and Tinea corporis was diagnosed in each of the remaining 3 subjects.

Penile ulcer, inguino scrotal swelling and squamous cell carcinoma of the penis were diagnosed in each of the 3 (6%) study subjects and all were male patients.

11 patients in the study had non-specific systemic complaints like malaise, anorexia, weight loss, generalized weakness etc. where a specific diagnosis of the organ system involved could not be made. Most appropriate explanation for these symptoms in the patients could be either the progression of disease process and/ or anemia. However, the average Hemoglobin concentration of this study group was 11.78 g/dL.

Organ System Involved: Correlation between TLyC & CD4 cell count

Table 20: Correlation Analysis between TLyC & CD4 cell count

Organ System Involved	Mean Hb	Mean TLC	Mean TLyC	Mean CD4 cell count	r Hb & CD4	r TLyC & CD4
Respiratory System	11.71	8829	1418.93	247.78	0.464	-0.127
GI System	11.43	9877.39	1427.46	268.48	-0.241	0.178
Nervous System	10.91	7500	943	94.93	0.672	0.811*
Dermatologic	12.66	7090	2160.20	350.73	0.046	-0.599
Genitourinary System	10.57	7433.33	1359.67	360.05	-0.487	-0.154
Miscellaneous	11.78	7488.92	1371.61	272.19	-0.35	0.657*

*. Correlation is significant at the 0.05 level (2-tailed).

Analysis done to study the correlation between TLyC and CD4 cell count for patients who were diagnosed to have different organ system involvement showed the following results:

- For the 14 patients who were diagnosed to have respiratory system involvement the mean Hb was 11.71 g/dL (Range: 7.3-17.0 g/dL). Mean TLC for this group of patients was 8829 cells/cumm (Range: 2800 – 18029 cells/cumm) and the mean TLyC was 1418.93 cells/cumm (Range: 630-3680 cells/cumm). The CD4 cell count ranged from 19-1591 cells/cumm with an average 247.78 cells/cumm. No significant correlation could be established between mean Hb and CD4 cell count (r = 0.464), but TLyC and CD4 cell count for this group by Pearson correlation showed some correlation (r = -0.127).
- About 20 patients presented with gastrointestinal system involvement, and mean Hb were 11.43 g/dL (Range: 7.8-18.3 g/dL) with a mean TLC 9877.39 cells/cumm (Range: 4300-25200 cells/cumm) and TLyC averaged 1427.46 cells/cumm (Range: 268-3680 cells/cumm). The CD4 cell count among these patients ranged between 18-1194

cells/cumm with a mean value, 268.48 cells/cumm. Again no significant correlation was seen between CD4 cell count and TLyC.

- Also, the 7 patients who had neurological illness on diagnosis had no significant correlation between CD4 cell count (Mean: 94.93 cells/cumm, Range: 18-249 cells/cumm) and Hb (Mean: 10.91 g/dL, Range: 9.0-15.9 g/dL). A significant correlation existed between CD4 cell count and TLyC (Mean: 943 cells/cumm, Range: 268-1526 cells/cumm) in these individuals ($r = 0.811$ at $p = 0.05$ level).
- No correlations were seen between CD4 cell count and Hb and TLyC among the group of patients with dermatologic conditions at diagnosis. The mean Hb for these patients was 12.66 g/dL (Range: 9.0-16.0 g/dL) and mean TLC was 7090 cells/cumm (Range: 5900-8350 cells/cumm). The mean TLyC and mean CD4 cell count were 2160.20 cells/cumm (Range: 948-4008 cells/cumm) and 350.73 cells/cumm (Range: 105-624 cells/cumm) respectively.
- Similarly, for the 3 subjects with genitourinary conditions no significant correlation for CD4 cell count seen with Hb and TLyC the results of which are shown in the table.
- Among the other group of patients in whom a specific diagnosis could not be arrived and who had nonspecific complaints of constitutional symptoms there was a significant correlation ($r = 0.657$) between TLyC and CD4 cell count at $p = 0.05$ level interval.

7. Laboratory Markers

All the patients included in the study were subjected to routine investigations like complete hemogram, urine examination and chest X-ray. Relevant clinical investigations like ultrasonography abdomen, liver function tests, renal function tests, Computerized tomography (CT) brain were carried out in certain patients according to the clinical situation. Abnormal findings were identified and relevant data were pooled and analyzed using descriptive statistics and correlation studies.

Here, Hb, TLC, ESR, Peripheral smear, Chest X-ray findings and CD4 cell count were tabulated and studied. Subgroup analysis were carried out for Hb, TLC and CD4 cell count for better understanding and identification of any significant findings.

7.1 Hb – Correlation between TLyC & CD4 cell count

Table 21: Hb Subgroup Analysis & Correlation for TLyC & CD4 cell count

Hb Class Interval	Sub Group	N (Sample Size)	Mean Hb	TLyC	CD4 cell count	r Hb & CD4	r TLyC & CD4
6-9.9	Total	16	8.87	1359.94	263.57	-0.033	0.131
	Male	9	8.84	1451.66	135.64	0.124	0.109
	Female	7	8.90	1242	428.05	-0.162	0.382
10-11.9	Total	11	10.75	1905	175.82	0.907**	0.573
	Male	6	10.57	1728.17	124.69	0.759	0.149
	Female	5	10.96	2117	237.16	0.945*	0.803
12-13.9	Total	11	12.94	2275.91	425.19	0.546	0.516
	Male	6	12.95	1997.67	336.63	0.084	0.514
	Female	5	12.92	2609.80	531.47	0.976**	0.384
14-15.9	Total	7	15.10	1419.50	451.55	0.355	-0.728
	Male	6	14.97	1439.40	493.97	0.541	-0.784
	Female	1	15.90	1300	197	a	A
16.0 & Above	Total	5	16.92	2491.20	132.49	-0.633	0.912*
	Male	5	16.92	2491.20	132.49	-0.633	0.912*
	Female	0	0	0	0	a	A
Total Group	Total Group	50	11.79	1805.70	295.23	0.123	0.127
	Total Male	32	12.35	1766	237.97	0.259	-0.008
	Total Female	18	10.98	1868	390.92	0.078	0.348

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

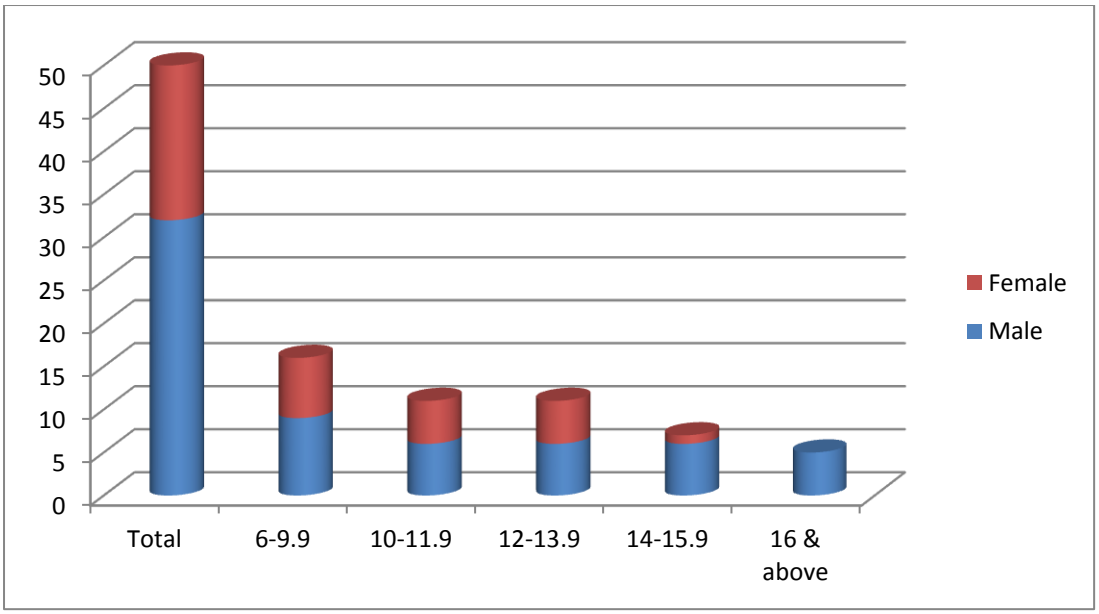

Fig.17: Hb Class Interval Distribution among Males & Females

Hemoglobin estimation was done for all the 50 patients included in the study and the data was compiled and a class interval distribution was done to study the descriptive statistics and to determine any correlation between TLyC and CD4 cell count for individual classes.

Of the total 50 patients 16 (32%) had Hb levels below 10 g/dL. 22 (44%) patients had Hb levels between 10-14 g/dL. For all the classes males had slightly lower mean Hb levels as compared to females and the mean TLyC and CD4 cell count were higher for female patients than the male counterparts. Of the 13 patients who had Hb levels more than 14 g/dL they were predominantly males i.e. 12 (24%) male patients. The mean TLyC and CD4 cell count for these 13 patients were 1965.20 cells/cumm and 164.75 cells/cumm respectively.

Pearson correlation study showed no significant correlation between TLyC and CD4 cell count for Hb classes between Hb 6-14.9 g/dL. However, for the 5 male patients with Hb levels above 16 g/dL correlation was significant at $p = 0.05$ level ($r = 0.912$).

Correlation for Hb levels and CD4 cell count showed only scattered weak correlation for individual subgroups. The correlation was significant at $p = 0.01$ levels for all the patients with Hb levels between 10-11.9 g/dL ($r = 0.907$) and it was significant at $p = 0.05$ levels for female subgroup of the same class ($r = 0.945$). A similar weak positive correlation at $p = 0.01$ levels for the female patients with Hb levels between 12-13.9 g/dL was observed ($r = 0.976$).

7.2 ESR – Correlation between TLyC and CD4 cell count.

Table 22: ESR – Correlation between TLyC & CD4 cell count

	Mean ESR	Mean TLC	Mean TLyC	CD4 cell count	r TLyC & CD4
Total (37)	56.16	9132.89	1747.24	292.13	0.078
Male (25)	48.28	9374.00	1844.56	249.78	-0.038
Female (12)	68.08	8833.33	1667.75	401.79	0.328

ESR report was available for a total of 37 out of 50 patients included in the study. The ESR values ranged from 5 mm/hour to 130 mm/hour with an average value of 56.16 mm/ hour. The corresponding mean TLC and TLyC values were 9132.89 cells/cumm (Range: 2800 – 25200) and 1747.24 cells/cumm (Range: 268 – 4830) respectively. The mean CD4 cell count was 292.13 cells/cumm (Range 18 - 1591). Pearson correlation for TLyC and CD4 cell count for the 37 subjects with respect to ESR was not significant (r = 0.078).

A sub group analysis was done to study any differences in ESR pattern among male and female patients. Although, the mean ESR was higher for the 12 female patients (68.08 mm/hour), on the contrary the mean CD4 cell count was higher for this group (401.79 cell/cumm). The corresponding mean ESR and CD4 cell count for the 25 male patients were 48.28 mm/hour and 249.78 cells/cumm respectively. No significant differences were noticed in the mean TLC and TLyC values in either sex.

8 patients in the study had ESR values greater than 100 mm/hour with 5 males and 3 female patients. 6 of these patients were suffering from respiratory illnesses and pneumonia was the common diagnosis made in them. All these 6 patients had CD4 cell

count less than 100mm/hour. The other 1 patient had tubercular lymphadenitis and the left 1 patient presented with fever with no other systemic involvement. The mean CD4 cell count for the patient with fever was 1194 cells/cumm. The TLC and TLyC values for these 8 patients ranged between 2800 cells/cumm and 11200 cells/cumm respectively, and, the mean values were 7250 cells/cumm and 1006 cells/cumm. The computed p value failed to establish any relationship between TLyC and CD4 cell count for these 8 patients ($r = 0.491$).

7.3 Peripheral Smear

Peripheral smear report was obtained in 28 patients. Of this 8 (28.57%) patients had normocytic normochromic anemia with a mean Hb of 9.25 g/dL. The CD4 cell count for all these patients were below 200 cells/cumm. The TLC and TLyC values were within the normal limits for these patients.

Microcytic hypochromic blood picture was seen in only 3 patients and there was no significant observation made in the TLC, TLyC and CD4 cell count of these patients with regard to peripheral smear report.

A total of 5 patients had peripheral blood eosinophilia and 9 patients had thrombocytopenia. No other significant observations were made in the corresponding TLC, TLyC and CD4 cell count in these patients.

7.4 Chest X-Ray

Of the 15 patients who had definite radiological signs of respiratory system involvement, 13 (26%) were male patients. Pneumonia was the most common finding on the chest X-ray with 2 patients having pleural effusion and 1 diagnosed to have pulmonary tuberculosis. These patients had mean TLC of 8281.95 cells/cumm (Range: 2800-18029) and TLyC of 1261 cells/cumm (Range: 268 – 3173). The CD4 cell count ranged from 26 cells/cumm to 672 cells/cumm (Mean: 151.22 cells/cumm). About 13 patients had CD4 cell count less than 200 cells/cumm. p value between TLyC and CD4 cell count for patients with definite radiological features of respiratory illness was significant with $r = 0.599$ at $p = 0.05$ level.

7.5 TLC : Correlation between TLyC and CD4 cell count

The estimated TLC for 50 patients were analyzed by subgroup analysis based on sex and based on class interval distribution to study the correlation between corresponding TLyC and CD4 cell count.

Table 23: TLC Descriptive Analysis & Pearson Correlation Studies

	Mean TLC	Mean TLyC	Mean CD4 cell count	r TLyC & CD4
Total	9355.50	1802.80	292.03	0.126
Male	9046.80	1766	237.97	-0.008
Female	9904	1868	390.92	0.348

The TLC values in the study of 50 patients ranged from 2800 cells/cumm to 25200 cells/cumm. The corresponding mean values of TLyC and CD4 cells were 1802.80 cells/cumm and 292.03 cells/cumm respectively. The p value (r = 0.126) did not show any significant correlation between TLyC and CD4 cell count. Further subgroup analysis was done for both the sex. The mean TLC and mean TLyC did not show significant changes from the total mean. But, the mean CD4 cell count for females (390.92 cells/cumm) was higher than that of males (237.97 cells/cumm). p value estimation between TLyC and CD4 cell count for either sex was not significant.

The TLC data of the study was analyzed by class interval distribution to understand any changes in the correlation patterns.

Table 24: TLC Class Interval Distribution

TLC	Mean Hb	Mean TLyC	Mean CD4 cell count	r Hb & CD4	r TLyC & CD4
Below 5000	9.4	1066.80	125.06	-0.228	0.881*
5000-10000	11.75	1811.00	300.74	0.471**	0.080
10000-15000	13.29	1533.00	338.99	-0.487	0.384
Above 15000	12.94	2468.70	328.90	-0.663	-0.099

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The TLC counts of all the 50 subjects were subjected to sub group analysis as above. The obtained values were distributed into 4 classes starting from those with counts less than 5000 cells/cumm to those with counts greater than 15000. The mean Hb, mean TLyC, mean CD4 cell count were calculated for each corresponding group and Pearson correlation was done to determine any correlation between Hb and CD4 cell count, and, TLyC and CD4 cell count for each class. The results were presented in the Table as shown above.

It was observed that for the group with TLC below 5000 cells/cumm, the mean Hb and mean TLyC were 9.4 g/dL and 1066.80 cells/cumm. The mean CD4 count for this group was less than 200 cells/cummat 125.06 cells/cumm. p value (0.881) for TLyC and CD4 cell count was significant at 0.05 level. No significant correlation existed between Hb and CD4 cell count for this group.

For the rest of the groups i.e. TLC between 5000-10000, 10000-15000 and above 15000 the computed p value for TLyC and CD4 cell count were 0.080, 0.384 and -0.099

respectively and the correlation was not significant. It was also observed that the mean TLyC were higher for these 3 groups than the group with counts less than 5000 cells//cumm. The mean CD4 cell count remained more or less constant for all the 3 groups and it was considerably higher than the former group.

7.6 CD4 Cell count Subgroup Analysis and It's Correlation with TLyC

Table 25: CD4 Cell Count Subgroup Analysis &It's Correlation with TLyC

CD4 Cell count	Mean Hb	Mean TLyC	r Hb & CD4	r TLyC & CD4
Total	11.98	1820.30	0.139	0.141
Less Than 200	11.64	1526.60	0.184	0.498**
200-500	12.45	2530.45	-0.281	0.007
Above 500	12.59	1945.00	0.080	-0.551

**. Correlation is significant at the 0.01 level (2-tailed).

In this method the obtained CD4 cell count of all the 50 subjects were classified into those with counts less than 200 cells/cumm, those with values falling between 200-500 cells/cumm and finally the group with counts greater than 500 cells/cumm. The mean Hb and mean TLyC were estimated for each group and group wise p values were computed to study correlation between Hb and CD4 cell count, and, TLyC and CD4 cell count.

30 (60%) study subjects had CD4 cell count less than 200 cells/cumm averaging 99.31 cells/cumm. The group had mean Hb and TLyC less than the corresponding total mean. A significant correlation at p = 0.01 level existed between TLyC and CD4 cell count for this group (r = 0.498).

For the other 2 groups the mean Hb and TLyC levels were comparatively higher than the total mean and no significant correlation existed between TLyC and CD4 cell count.

7.7 TLyC & CD4 cell count – XY Scatter Plot

The two key variables of the study TLyC and CD4 cell count were plotted using XY scatter plot to study the correlation between them.

In the Fig.18 CD4 cell count was kept as the control parameter (or independent variable) and TLyC was used as the dependent variable.

The Fig.19 TLyC was used as the control parameter and CD4 cell count as the dependent variable.

In both the scenarios it was observed that for low CD4 cell count and for low TLyC counts, the two parameters had a reasonable correlation between them ($r = 0.4757$, $p = 0.0005$). But in either case with the higher values of either parameter there existed no correlation between the two parameters.

Table 26. Correlation Coefficient at CD cell count <200 cells/cumm

Sample size	50
Correlation coefficient r	0.4757
Significance level	P=0.0005
95% Confidence interval for r	0.2274 to 0.6659

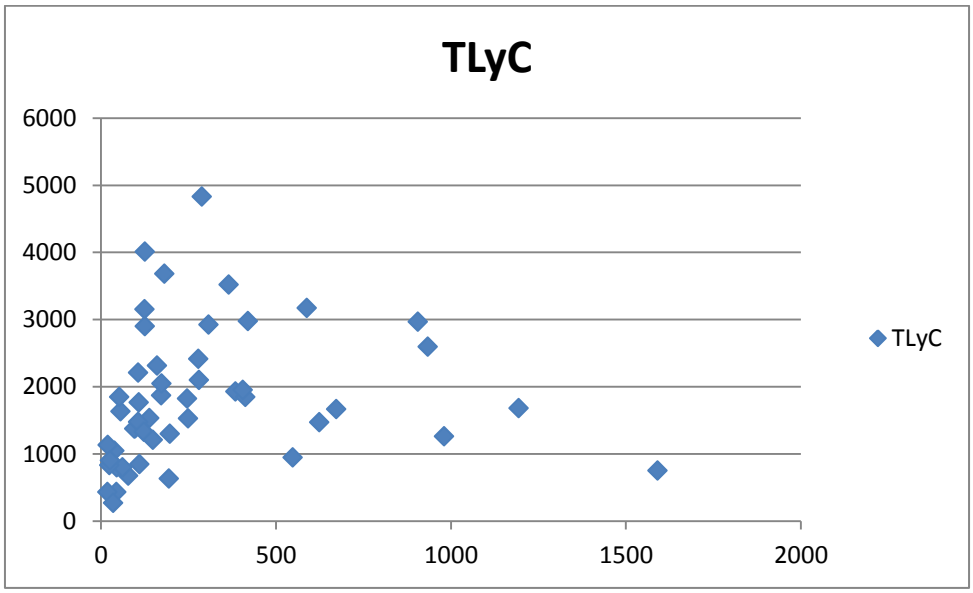

Fig.18: XY Scatter Plot with TLyC along Y axis

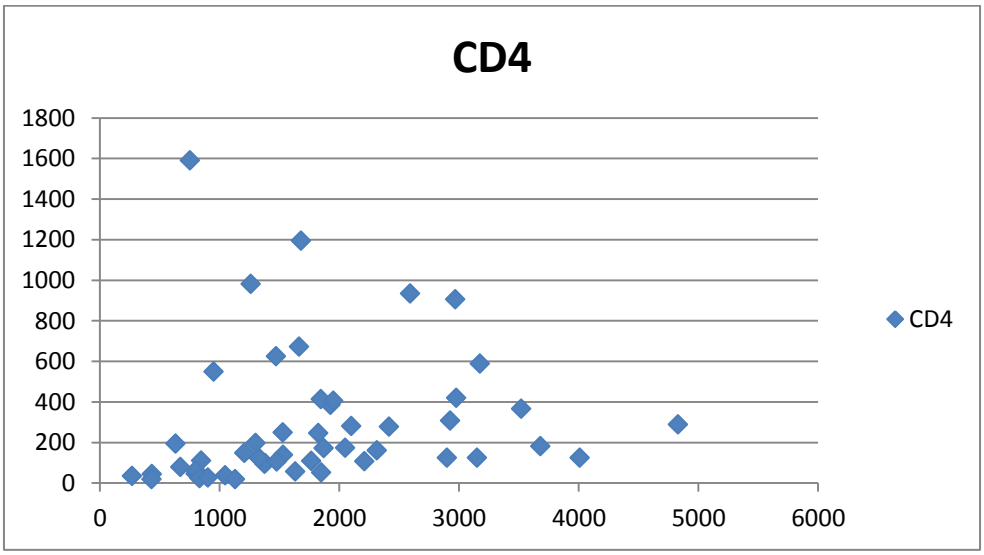

Fig.19: XY Scatter Plot with CD4 cell count along Y axis

7.8 CD4 Cell count and TLyC – ROC Curve

An ROC Plot was drawn between TLyC and CD4 cell count to establish the sensitivity and specificity of TLyC with respect to changes of CD4 cell count. Here TLyC was chosen as the variable and a cut off of Cd4 cell count </= 200 cells/µL was chosen to calculate the sensitivity and specificity. The cut off value was chosen from the observations of XY scatter plot and correlation analysis of CD4 cell count and TLyC.

Table 27. ROC Variables

Sample size		50
Positive group :	CD4 = 1	30
Negative group :	CD4 = 0	20

Table 28. ROC – Area Under Curve

Area under the ROC curve (AUC)	0.723
Standard Errora	0.0730
95% Confidence intervalb	0.579 to 0.840
z statistic	3.060
Significance level P (Area=0.5)	0.0022

a DeLong et al., 1988
b Binomial exact

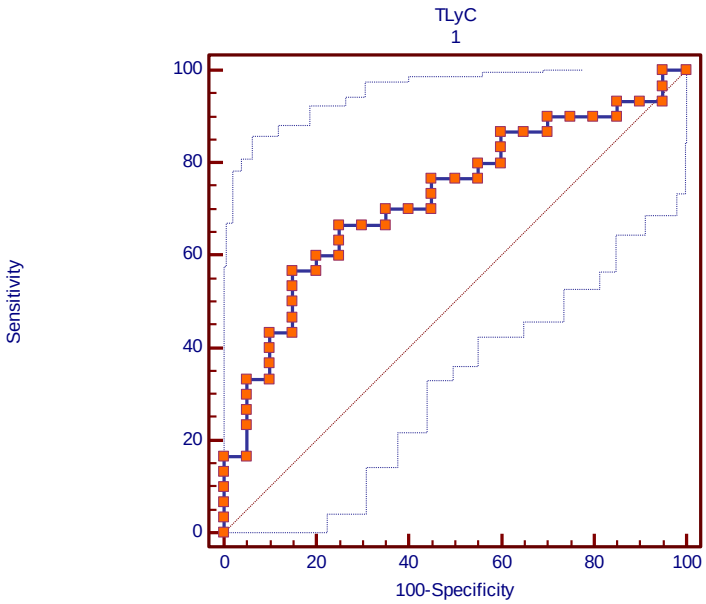

Fig. 20. ROC Graph

Table 29.Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
< 268	0.00	0.0 - 11.6	100.00	83.2 - 100.0		1.00
<=672	16.67	5.6 - 34.7	100.00	83.2 - 100.0		0.83
<=752	16.67	5.6 - 34.7	95.00	75.1 - 99.9	3.33	0.88
<=902	33.33	17.3 - 52.8	95.00	75.1 - 99.9	6.67	0.70
<=948	33.33	17.3 - 52.8	90.00	68.3 - 98.8	3.33	0.74
<=1206	43.33	25.5 - 62.6	90.00	68.3 - 98.8	4.33	0.63
<=1260	43.33	25.5 - 62.6	85.00	62.1 - 96.8	2.89	0.67
<=1380	56.67	37.4 - 74.5	85.00	62.1 - 96.8	3.78	0.51
<=1472	56.67	37.4 - 74.5	80.00	56.3 - 94.3	2.83	0.54
<=1475	60.00	40.6 - 77.3	80.00	56.3 - 94.3	3.00	0.50
<=1526	60.00	40.6 - 77.3	75.00	50.9 - 91.3	2.40	0.53
<=1632*	66.67	47.2 - 82.7	75.00	50.9 - 91.3	2.67	0.44
<=1680	66.67	47.2 - 82.7	65.00	40.8 - 84.6	1.90	0.51
<=1764	70.00	50.6 - 85.3	65.00	40.8 - 84.6	2.00	0.46
<=1845	70.00	50.6 - 85.3	55.00	31.5 - 76.9	1.56	0.55
<=1870	76.67	57.7 - 90.1	55.00	31.5 - 76.9	1.70	0.42
<=1950	76.67	57.7 - 90.1	45.00	23.1 - 68.5	1.39	0.52
<=2048	80.00	61.4 - 92.3	45.00	23.1 - 68.5	1.45	0.44
<=2100	80.00	61.4 - 92.3	40.00	19.1 - 63.9	1.33	0.50
<=2312	86.67	69.3 - 96.2	40.00	19.1 - 63.9	1.44	0.33
<=2592	86.67	69.3 - 96.2	30.00	11.9 - 54.3	1.24	0.44
<=2898	90.00	73.5 - 97.9	30.00	11.9 - 54.3	1.29	0.33
<=2975	90.00	73.5 - 97.9	15.00	3.2 - 37.9	1.06	0.67
<=3150	93.33	77.9 - 99.2	15.00	3.2 - 37.9	1.10	0.44
<=3520	93.33	77.9 - 99.2	5.00	0.1 - 24.9	0.98	1.33
<=4008	100.00	88.4 - 100.0	5.00	0.1 - 24.9	1.05	0.00
<=4830	100.00	88.4 - 100.0	0.00	0.0 - 16.8	1.00	

The above analysis shows that when the CD4 cell count is taken at a cut off of </=200 cells/µL to study the sensitivity and specificity of TLyC in predicting the immune status, then at mean TLyC value </= 1526 cells/µL the sensitivity and specificity are 60% and 75% respectively. The area under the curve is 0.723.

DISCUSSION

CD4 cell, a T lymphocyte subset is the major immune effector cells in the body. Infection with HIV virus mainly attacks and destroys CD4 cells, resulting in quantitative decrease and functional depletion of CD4 cells[82], causing various opportunistic infections and tumors, and leading to death of the affected individual. Though clinical assessment remains the most essential basis for monitoring HIV infection, it lacks sensitivity in determining both disease stage and progression and, it is used in conjunction with laboratory measures.[83] Among the wide varieties of surrogate markers available CD4 lymphocyte count has been widely used as the marker of choice in HIV infected individuals[74] and is currently recommended by WHO[78]. CD4 monitoring of HIV infected individuals is required to decide on initiating HAART, and for deciding on the treatment of opportunistic infections.[84-88] However, CD4 monitoring requires expensive equipment, reagents and skillful staff which are not available at resource-limited settings.[78, 89] This necessitated the need for low cost alternative surrogate markers in resource-limited settings to monitor disease progression in HIV infected individuals. WHO guidelines suggest the use of simple laboratory investigations like hemoglobin (Hb) of <12 g/dl and Total Lymphocyte Count (TLyC) of <1,200 cells/μL in individuals in stage II or III of the disease, as surrogate markers.[90] Several studies have been conducted across different settings to establish the correlation between CD4 cell count and TLyC and other surrogate markers as a suitable alternative surrogate markers to CD4 cell count in monitoring HIV disease progression and response to HAART therapy. The results have been varied across different centers. Some studies have established a high consistency between CD4 cell count and TLyC[91] while others have contradicted such a correlation[92]. A few others have demonstrated high consistency between CD4 cell count and TLyC in patients with advanced state of immunosuppression.[93-96]The differences on the validity of surrogate markers could be due to many reasons like patient demographics, mode of acquisition,

presence or absence of other infections, epidemics or chronic debilitating illnesses and adherence to treatment.

This study was conducted at a rural tertiary care center to understand the correlation between CD4 cell count and TLyC with respect to clinical profile of newly diagnosed HIV patients admitted in the hospital and its applicability as an alternative surrogate marker to CD4 cell count in deciding initiation of HAART and treatment for opportunistic infections. The results obtained are compared with other studies conducted in this discipline and a final conclusion is drawn at the end.

The current study showed that HIV affected predominantly the individuals in the age group 20-49 years, constituting 84% of the total study subjects. Further, incidence among the males (64%) was higher than that of females with a male to female ratio of 1.78:1. The findings were similar to that found in other studies done in different parts of the country and neighboring countries.[97-98]The higher incidence in males is mainly because of the societal culture where males are the bread earners of the family and females are largely restricted to household work thus exposing males to greater number of risk factors. In this study, over 80% female patients were homemakers. Most of the females had acquired the infection through their husband, who had in turn acquired it from commercial sex workers; a finding which was consistent with other studies in India.[99-101] None of the patients in the study had homosexual behavior or injection drug abuse. This study showed that the HIV infection is no longer restricted to wandering individuals like those in transport department, but is also prevalent among other occupations like manual labors, agriculturist and other low risk groups. Singh R et al., 2009[102] showed similar findings in their study. Smoking and alcoholism were identified as additional risk factors in over 90% of male subjects in this study. 65.66% of the individuals had either smoking or alcoholism as additional risk factors and 25% of subjects had both. A systematic

review of studies indicated that smoking might be independently associated with acquiring HIV infection i.e. increased risk of HIV sero-conversion but not to progression to AIDS[103]. Similarly, studies have shown that alcohol consumption is again linked with increased risk of HIV transmission. In some studies, the prevalence rate of alcohol use in the HIV-infected population is high, almost twice those found in the non-HIV-infected population.[104]Though smoking and alcohol consumption often seem to be missed as a subtle problem, but they can increase HIV transmission rate and alter the disease progression and treatment outcome in the following ways:

- Diminishing adherence to medications
- Increasing risk of hepatic injury
- Reducing the patient's ability to practice safer sex[105,106]
- Increasing the risk of side effects from medications
- Changing pharmacokinetics of prescribed drugs

Based on the above demographic and personal profile, a correlation study was done to study the correlation between TLyC and CD4 cell count in either sex and with respect to age. It was found that the female subjects had a higher mean TLyC (1783.79 cells/cum) and CD4 cell count (419.01 cells/cumm) as compared to the male subjects, an indirect indication of the later contact of infection by females. There was weak correlation between TLyC and CD4 cell count for both male and female subjects over 50 years (r= 0.993 and r=1.000 at p = 0.01 level respectively). The small sample size of these groups of patients made it difficult to draw a significant conclusion. To conclude, there was no significant correlation between TLyC and CD4 cell count with respect to age and sex. Further the lower mean values amongst the males are due to the effect of multiple factors

potentiating the immune suppressive effect of HIV, like cigarette smoking and alcohol consumption.

Poverty, illiteracy and lack of awareness are playing a major role in the spread of HIV in this region affecting the economically productive and sexually active group.

The symptomatology of the HIV positive individuals at initial presentation was varied in this study ranging from fever, and constitutional symptoms to the more devastating ones with CNS involvement. Fever (38%) was the most commonly reported symptom at the first interview of most individuals. Others were abdominal pain (8%), nausea and vomiting (26%), cough (26%) and constitutional symptoms (10%). CNS symptoms, genital ulcers and other dermatological complaints were also observed in this study, but with a comparatively lower frequency. Tuberculosis, Herpes zoster and oral candidiasis were opportunistic infections diagnosed among the patients in this study.[97, 107] These opportunistic infections occurred in all the individuals who had initial CD4 cell count <200 cells/cumm.[108]

The clinical presentation of HIV varies from patient to patient and from country to country. This is influenced by several factors such as baseline health and nutritional status, environment, endemic diseases, and access to therapy. A clinical examination of patients in the study showed fever, pallor, lymphadenopathy, and skin and mucosal changes to be the most common findings present. These symptoms were analyzed in accordance with Hb%, TLyC and CD4 cell count to see the accuracy of these clinical markers in predicting the disease stage and need for HAART. About 20 patients (40%) on examination had fever. 19 patients (38%) had pallor which was confirmed by further laboratory investigations with 84.21% having normocytic normochromic anemia with or without thrombocytopenia. In this study it was observed that neither Hb% nor TLyC

could be used as surrogate marker to CD4 cell count in patients with fever or pallor for determining the stage of HIV infection and on initiating ART. HIV produces a variety of clinical cutaneous manifestations. As the CD4 cell count decreases, the severity of the skin condition increases with frequent relapses. An American study done to understand the correlation between skin disorders and CD4 cell count is illustrated below in the figure below [109]

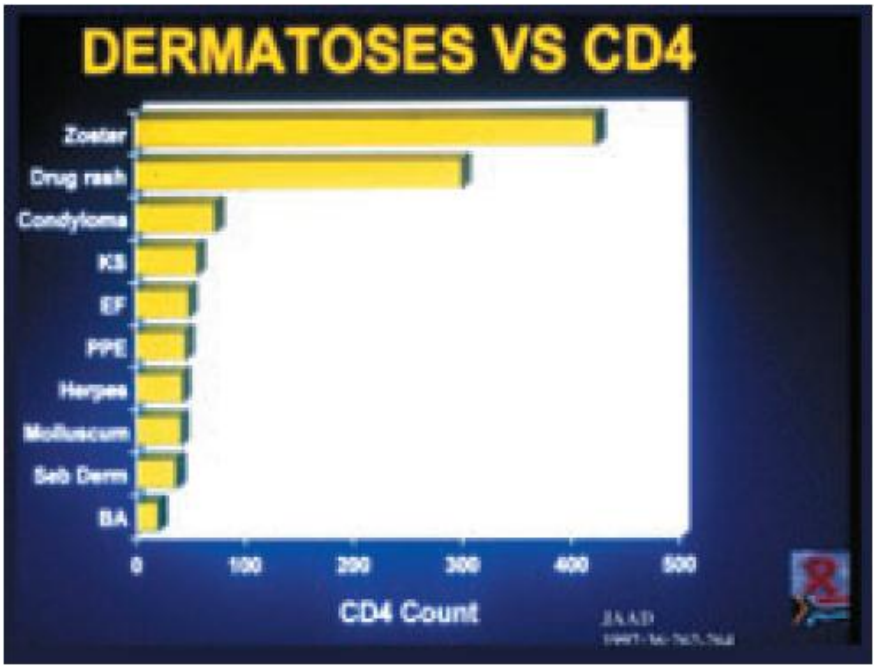

Fig.21: Correlation between mean CD4 cell count and incidences of specific skin disorders in patients with HIV infection.109

In this study xerosis of skin was the most common skin finding present in 24 of 26 patients with skin changes in HIV who had mean CD4 cell count >200 cells/cumm. Mucocutaneous and herpes zoster were reported in the other two patients who had CD4 cell count <200 cells/cumm. Sengupta et al [110]showed oral candidiasis to be the predominant oral lesion present in about 36% of the patients. There was no significant correlation between TLyC and CD4 cell count in the study to predict the stage of HIV for the patients with skin manifestations although presence of mucocutaneous candidiasis and herpes zoster did indicate severe immunocompromised state in these individuals. TLyC was a poor predictor of immunocompromised state in the individuals with skin manifestations. The above findings were consistent with the observation made by Singh et al[102] in their study.

Lymphadenopathy is common in HIV infection and it could be either due to infection, malignancies or due to the HIV virus itself. Studies conducted in India by Shobanaet al111 and Lakshmi et al[112] showed that the most common form of lymphadenopathy diagnosed by FNAC were reactive lymphadenitis, tubercular lymphadenitis and lymphomas. In the present study, it was observed that 9 patients had reactive lymphadenitis and 1 patient had tubercular lymphadenitis. These patients had a mean CD4 cell count, 145.21 cells/cumm, which was significantly lower than the patients with no lymphadenopathy. To conclude, lymph node involvement was common among subjects with CD4 cell count <200 cells/cumm and TLyC was correlated with CD4 cell count ($r = -0.029$) in predicting the state of immunosuppression in patients with lymphadenopathy.

The final diagnoses made in this study were categorized according to the predominant system involvement and the pattern of TLyC and CD4 cell count was studied in them. Respiratory system and Gastrointestinal system were the most common organ systems

involved in the study. HIV patients usually present with the usual symptoms but have a more insidious course as compared with other immunocompromised patients.[113] Majority of the patients in the current study had fever (38%) as the initial presentation. Cough, expectoration and breathlessness were the predominant pulmonary symptoms, and, nausea, vomiting and pain abdomen were the predominant symptoms in patients with gastrointestinal system involvement. A diagnosis of pulmonary tuberculosis was made in 4 patients (2% of the total) and Pneumocystis jerovecii pneumonia in 1 patient. In India, the most common opportunistic pulmonary infection among the HIV positive individuals is pulmonary tuberculosis.[114] Understanding the HIV-TB co-infection is of great importance because of its increasing prevalence, severity of clinical presentation and rapidity of progression of illness. Tuberculosis can occur over a wide range of CD4 counts, but is more frequent at counts <300 cells/cumm.[114] Chest radiographs may show middle and lower lobe infiltrates, military TB, tubercular pneumonia, and hilar or mediastinal lymphadenopathy[115] with or without sputum positivity. Extra-pulmonary TB has been reported in many organs: lymph nodes (most common), spleen, liver, bone, bone marrow, heart, central nervous system, gastrointestinal tract, kidneys, adrenals, thyroid, and prostate.[116] Occurrence of Pneumocystis jerovecii pneumonia establishes the diagnosis of AIDS.[117] In India, its occurrence is very low (0.7% - 7%) and occurs in patients with CD4 cell count <200 cells/cumm.[116, 118]In the Indian context, PCP can simultaneously occur with other pulmonary infections, including TB, cryptococcosis, and cytomegalovirus.[119]In the current study, the prevalence of pneumocystis jerovecii pneumonia was 2%. Among the patients with respiratory system involvement the mean CD4 cell count was >200 cells/cumm and TLyC was a poor predictor of immune status of the affected individuals (r = -0.127).

Diarrheal illnesses are the most common gastrointestinal manifestations among the AIDS patients, affecting up to 76% of those with AIDS.120 Independent reports from India have shown that Isospora belli and Cryptosporidium parvum were the most common causes of chronic diarrheal disease in AIDS patients.[121,122] Other bacterial and viral agents have also been described but with a lower frequency. Candidal esophagitis causing dysphagia and odynophagia is also common among patients with advanced HIV infection. In the current study, gastrointestinal system involvement was seen in 40% of the cases and diarrheal illnesses were most common among it. Although an exact etiologic diagnosis of diarrhea could not be established most were infective etiology on stool examination. The mean TLyC and mean CD4 cell count in these patients were 1427.46 cell/cumm and 268.48 cells/cum respectively. Correlation between TLyC and CD4 cell count showed that TLyC was not a suitable surrogate clinical marker to determine the state of immunosuppression among these patients (r = 0.178).

Neurological complications of HIV disease can be seen in 20% of outpatients in HIV clinics and almost half of HIV patients being treated as inpatients.[123]The spectrum of neurological conditions includes, opportunistic infections, malignancy, AIDS related dementia, and vasculitis/stroke. Cryptococcal meningitis (CM) has been reported as the most common opportunistic infection of the CNS of Indian patients with HIV.[123-125]It accounted for 2-4.7 per cent of all opportunistic infections in two large HIV-positive patient cohorts in Mumbai and Chennai.[107,108] In southern Indian patients, diagnosis of CM was associated with a 7-fold increase in risk of death and the median CD4 count at presentation was 91 cells/μl.[107,108]Clark RA et al[126]observed that the presenting clinical features of Cryptococcal meningitis were often subtle and nonspecific and included malaise, fever, nausea, vomiting and head ache in 75% to 90% of the patients. Tubercular meningitis is less common than Cryptococcal meningitis and in a cohort of patients

accounted for only 18% of patients diagnosed with neurological complications.[123]Stephen J. Mcphee Maxine A. Papadakis[127]studied the relationship of CD4 counts to development of opportunistic infections. When the CD4 counts vary between 200-499/cumm, opportunistic infections were reported eg. Toxoplasmosis, and Cryptococcosis.[127] When the CD4 counts <50/cumm, opportunistic infections reported were disseminated MAC infection, CMV retinitis and CNS lymphoma.[127] Among the 7 patients in the study with neurological manifestations Cryptococcal meningitis and Tuberculous meningitis were diagnosed in 2 patients each (28.56%). The mean TLyC and CD4 cell count in these patients were 943 cells/cumm and 94.93 cells/cumm respectively with a significant correlation (r = 0.811) between the two parameters. The patients with neurological complications presented in an advanced state of immunosuppression and TLyC served as a suitable marker to CD4 cell count among these patients.

Cutaneous manifestation of HIV can occur in up to 90% patients[127] and many can be the initial presenting symptom of HIV infection and are classified into five groups: infectious, autoimmune, drug-induced, HIV related and malignancies. Herpes zoster and cutaneous and mucocutaneous candidiasis occur early in the course of HIV infection and represent a state of advanced immunosuppression. Oral candidiasis has been reported in up to 70% of the cases with median CD4 cell count ranging from 107 and 189 cells/cumm in different studies.[107,108] 8% of the patients with HIV had herpes zoster with a median CD4 cell count of 250 cells/cumm.107Staphylococcal skin infection is the most common cutaneous bacterial infection in HIV patients. It was reported in 1.3 per cent of 833 HIV-positive Indian patients, and occurred at a mean CD4 count of 410 cells/µl.[128]In the present study, the common dermatologic conditions diagnosed were, herpes zoster, candidiasis and cellulitis of lower limb. The findings of these patients were consistent

with reports from other Indian studies.[107, 108, 127, 128] However, the TLyC did not correlate with CD4 cell count (r = -0.599) among these patients.

After having assessed the role of TLyC as a suitable surrogate marker to CD4 in HIV positive patients with respect to their clinical profile, the study was further extended to study any correlation between the two variables with respect to common laboratory markers in HIV patients. Hb%, ESR, Peripheral blood smear and Total Leucocyte Counts were used in the study and in the context of these laboratory parameters, applicability of TLyC as an alternative marker of CD4 cell count was studied. There have been different and occasionally contradicting reports on the validity of these tests as surrogates of CD4 cell count.

Some studies have reported that TLC <1200 cells/cumm and Hb <12 g/dL had a positive correlation with CD4 cell count <200 cells/cumm.[129-131] In the current study the Hb values for male and female patients averaged 12.35 g/dL and 10.98 g/dL and overall it correlated poorly with CD4 cell counts for predicting the state of immunosuppression. Further subgroup analysis of either sex showed only weak correlation with CD4 cell count. Also, in these groups of patients TLyC values correlated poorly with CD4 cell count as a surrogate marker for the latter in HIV. These findings were consistent with the study done by Alaviet al.[78] This disagreement may be due to socio-economic status, malnourishment, and others prevalent in the rural areas.

Similarly, the ESR values were categorized according to sex and ESR levels and it was found that very high ESR values were suggestive of high prevalence of tuberculosis, the most common opportunistic infection in HIV patients. But TLyC values did not correlate with CD4 cell count even in this group. In the patients with anemia, the most common peripheral smear finding was normocytic normochromic blood picture with or without

thrombocytopenia. Laboratory value of Hb <10 g/dL with confirmed peripheral smear report suggested advanced state of immunosuppression with mean CD4 cell count of <200 cells/cumm. Whether this effect was to the malnourishment, opportunistic infections or due to HIV infection itself the cause – effect relationship could not be established. The current study showed that patients who had definite radiological signs of respiratory illness like pneumonia, tuberculosis etc. had a mean CD4 cell count <200 cells/cumm and the correlation between TLyC and CD4 cell count stood significant with r = 0.599 (at p = 0.05 level).

The present study showed that the CD4 cell count of males was lower than that of females. Further, this study supports the WHO recommendations of starting HAART in patients with WHO stage III of IV disease or stage II disease with an TLyC of <1200 cells /cumm irrespective of availability of CD4 cell count in resource limited settings.[132] The present study showed that in patients with TLC below 5000 cells/cumm, the mean TLyC was 1066.80 cells/cumm (i.e. <1200 cells/cumm) and the corresponding mean CD4 cell count was 125 cells/cumm with a significant correlation between TLyC and CD4 cell count (r = 0.881). A recent study by Kakkar et al from India reported similar findings and a positive agreement with WHO guidelines.[133] The study also analyzed the 30 patients with CD4 cell count less than 200 cells/cumm and found that these patients had a mean TLyC of 1526 cells/cumm and p value of 0.498 standing significant at 0.01 level similar to the results obtained in the study by Alavi et al. The sensitivity and specificity at the mean TLyC cut off value was 60% and 75% respectively. The area under the curve was 0.723. The XY scatter plot further suggests the positive relation between CD4 cell count and TLyC at values <200 cells/cumm and <1200 cells/cumm respectively.

TO SUMMARIZE, to the best of my knowledge this is the first study from South India where an attempt was made to correlate the CD4 cell count and TLyC with respect to clinical profile of newly diagnosed HIV positive patients in a resource limited rural tertiary care center. Some of the significant observations made in the study were;

- HIV affected predominantly the sexually active people in the age group 20—49 years with a decreasing incidence thereon. Males had higher incidence than females. Sexual mode of transmission was the most common mode and almost all males had history of unsafe sexual practice with a partner(s) other than his spouse, while females contracted the illness from their husbands.
- As earlier believed, it is no longer a disease of the vagabonds on the travel and the disease had spread to involve people with other occupations as well. Cigarette smoking and alcoholism were additional risk factors observed predominantly among the males.
- Overall, females had higher mean CD4 cell count and TLyC than males with age having only a weak effect on the correlation between CD4 cell count and TLyC.
- Patients CNS manifestations at presentation, female patients with Low Hb values, patients with lymphadenopathy, and, patients with CX-Ray findings of pneumonia or tuberculosis had significant correlation between CD4 cell count and TLyC suggesting a possible role of these clinical parameters in addition to TLyC in deciding the state of immunosuppression and initiation of HAART and/or opportunistic infection prophylaxis.

CONCLUSION

CD4 cell count estimation is the standard measure of immunodeficiency in HIV infected individuals and helps monitor disease progression and initiate HAART. Non availability of this investigation in resource limited settings should not delay or postpone treatment of HIV infected individuals at appropriate time. This study shows that there is strong positive correlation between CD4 cell count (<200 cells/cumm) and TLyC. TLyC can be employed as a suitable surrogate marker to CD4 cell count in monitoring HIV individuals in resource constrained settings. Further, clinical parameters like pallor, lymphadenopathy, neurological manifestations at presentation, severe respiratory illnesses and other opportunistic infections along with laboratory parameters like Hb, Platelet count, peripheral smear reports, ESR and significant CX-ray findings could be utilized to device a clinical - laboratory criteria for rural settings to monitor disease progression.

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ANNEXURE

CORRELATION BETWEEN CD4 COUNT AND TOTAL LYMPHOCYTE COUNT WITH RESPECT TO CLINICAL PROFILE OF NEWLY DIAGNOSED HIV POSITIVE PATIENTS

PROFORMA

Name:

Age:

Sex:

Occupation:

Serial Number:

Date of Recording Data: Contact Number:

Chief Complaints:

History of Presenting Illness:

Past History: Medical

Surgical

Family History:

Personal History: Marital Status –

Diet –

Loss of Appetite –

Weight Loss –

Bowel & Bladder –

Smoking –

Alcohol –

Injection/ Drug Abuse –

High risk sexual behavior –

General Physical Examination:

Pallor: Temperature:

Nails: Pulse Rate:

Icterus: Blood Pressure:

Cyanosis: Respiratory Rate:

Pedal Edema:

Skin & Mucosal Changes:

Lymphadenopathy:

Chelosis:

Petechiae:

Purpura:

SYSTEMIC Examination:

Respiratory System –

Cardiovascular System –

Abdominal Examination –

Central Nervous System –

INVESTIGATIONS

HIV Status –

Complete Hemogram: Hb% -

RBC Count –

Total Leukocyte Count –

Differential Leukocyte Count – Neutrophils/ ,
Lymphocytes/

Eosinophils/ , Monocytes/

Basophils/

Total Lymphocyte Count –

Platelet Count –

ESR –

Peripheral Smear –

Chest X-ray:

USG Abdomen:

CSF Analysis:

Pleural Fluid Analysis:

Peritoneal Fluid Analysis:

Renal Function tests: Blood Urea - , Serum Creatinine –

Electrolytes: Na+ - , K+ -

Liver Function Tests –

Blood Culture & Sensitivity:

Urine: Routine –

Microscopy –

Culture & Sensitivity –

Sputum: Grahm Stain –

ZN Stain –

Culture & Sensitivity:

HBsAg:

VDRL:

Cytological Studies:

CT scan:

MRI Study:

CD4+ Counts:

CD8+ Counts:

Sl. No.	Sex	Age	Occupation	Marital Status	Chief Complaints	Risk Factor	Smoking	Alcoholism	Temperature	PR	BP	Pallor	Nails	Skin & Mucosa	Lymphadenopathy	Cyanosis	Pedal Edema
1	M	58	Merchantile	Married	Loose stools, nausea, vomiting, fever	Multiple partners	0	0	N	100	90/70	0	N	Xerosis	0	0	0
2	FM	47	Housewife	Married	Generalized weakness, ↓ appetite	Husband positive	0	0	N	82	124/70	0	N	Hyperpigmented macules	0	0	0
3	M	50	Coolie	Married	Fever, cough, expectoration	Multiple partners	1	1	N	98	120/80	0	Clubbing	Curdy white patch over tongue	1	0	0
4	FM	40	Housewife	Married	Routine Screening	Husband positive	0	0	N	78	120/70	0	N	Xerosis	0	0	0
5	M	45	Driver	Married	Pain abdomen, nausea, vomiting	Multiple partners	1	0	N	80	120/80	0	N	N	1	0	0
6	FM	30	Housewife	Married	Fever	Husband positive	0	0	F	80	100/60	1	Platynychia	Xerosis	1	0	0
7	M	38	Police	Married	Rash over abdomen	Multiple partners	0	1	N	78	130/80	0	N	Erythematous Rash - Herpes	0	0	0
8	M	34	Quarry	Married	Pain abdomen, altered sensorium	Multiple partners	1	1	N	60	140/100	1	N	N	0	0	0
9	M	27	Business	Married	Wt. loss, N&V, ↓appetite, cough with expectoration, PTB +ve		1	1	N	82	100/60	1	Clubbing	Xerosis, pustules +	1	0	0
10	FM	53	Housewife	Married	Fever, N&V, Loose stools, altered sensorium, k/c/o HTN		0	0	N	110	80/60	1	N	↓ Skin turgor, dry mucosa	0	0	0
11	M	40	Farmer	Married	Altered sensorium, k/c/o PTB (defaulter)	? Multiple partners	0	1	N	90	110/90	1	N	Xerosis	0	0	0
12	M	28	Mechanic	Married	Fever, Headache, ↓appetite, wt loss	Multiple partners	1	1	Febrile	92	120/70	0	N	N	1	0	0
13	FM	33	Housewife	Married	Fever, Cough with expectoration, loose stools	Husband Positive	0	0	Febrile	106	100/60	1	Platynychia	Xerosis	0	0	0
14	M	45	Farmer	Married	Loose stools, vomiting		0	0	N	84	110/70	1	Platynychia	Dry skin and mucosa, ↓skin turgor	0	0	0
15	FM	35	Housewife	Married	Fever, loose stools, vomiting	Husband Positive	0	0	N	80	80/60	1	Platynychia	Xerosis	0	0	0
16	M	42	Security Supervisor	Married	Fever, cough with expectoration, breathlessness	Multiple Partners	0	1	Febrile	90	80/40	1	Clubbing	Xerosis	0	0	0
17	M	50	Driver	Married	B/L pedal edema, fever, cough with expectoration	Multiple partners	0	1	N	78	130/80	1	N	Xerosis	1	0	1
18	FM	42	Housewife	Married	Mass per vagina	Husband Positive	0	0	N	86	120/80	0	N	N	0	0	0
19	M	45	Coolie	Married	Pain abdomen, loose stools	Multiple partners	1	1	N	82	120/80	1	N	Xerosis	0	0	0
20	M	26	Cab Driver	Single	Pain, itching and swelling over tip of penis	Multiple partners	1	1	N	90	120/70	0	N	N	0	0	0
21	FM	21	Housewife	Married	Sudden loss of vision in both the eyes	Husband Positive	0	0	N	74	100/60	0	N	N	0	0	0
22	FM	40	Farmer	Widow	Fever, vomiting	Husband positive	0	0	Febrile	92	140/90	1	N	Xerosis	1	0	0
23	M	28	Painter	Married	Fever with Skin Rash (Herpes), k/c/o Tb lymphadenitis	Multiple partners	1	1	Febrile	92	120/70	1	N	Xerosis, herpes zoster + over forehead	1	0	0
24	M	26		Married	L sided chest pain	Multiple partners	1	1	N	82	120/80	0	N	Xerosis	0	0	0
25	FM	28	Teacher	Married	Primipara with HIV positive status	Husband positive	0	0	N	80	120/80	0	N	N	0	0	0
26	FM	20	Housewife	Married	Primipara with HIV positive status	Husband positive	0	0	N	84	140/100	1	N	N	0	0	1
27	FM	50	Housewife	Married	Fever, Loose stools Vomiting	Multiple partners	0	0	N	80	140/70	0	N	Xerosis	0	0	0
28	M	46	Security Guard	Married	Abdominal pain, N&V	Multiple partners	0	1	N	78	120/80	0	N	N	0	0	0
29	FM	40	Housewife	Married	Routine screening	Husband positive	0	0	N	82	130/70	0	N	N	0	0	0
30	M	28	Driver	Married	Fever, Loose stools, N&V, pain abdomen	Multiple partners	1	1	Febrile	120	140/90	0	N	Xerosis	0	0	0
31	M	34	Driver	Married	Fever, running nose, cough with expectoration	Multiple partners	1	1	N	68	110/70	0	N	N	0	0	0
32	FM	36	Housewife	Married	P2L2D1	Husband positive	0	0	N	76	170/110	1	N	N	0	0	1
33	FM	30	Manual Labor	Married	P2L2	Husband positive	0	0	N	80	130/80	1	Platynychia	Xerosis	0	0	1
34	M	40	Driver	Married	Fever, cough with expectoration, vomiting	Multiple partners	1	0	Febrile	97	100/80	0	N	Xerosis	0	0	0
35	M	35	Farmer	Married	Cough with expectoration & breathlessness	Multiple partners	1	1	N	90	120/70	0	N	N	0	0	0
36	M	36	Chef	Married	Chest pain & giddiness	Multiple partners	1	1	N	80	100/70	0	Clubbing	N	0	0	0
37	M	48	Driver	Married	Exertional Dyspnea	Multiple partners	1	1	N	84	110/70	0	Clubbing	N	0	1	0
38	M	22	Manual Labor	Married	Ulcerative lesion over Lft. cheek with inability to open the mouth	Multiple partners	1	0	N	86	120/80	0	Clubbing	N	1	0	0
39	M	38	Conductor	Married	Fever, Swelling of scrotum & penis, Bluish discoloration of Lft groin & thigh, Loose stools	Multiple partners	0	1	N	92	110/80	0	N	Reddish discoloration over groin with ulcerations & sero sanginous discl	1	0	0
40	M	34	Driver	Married	Routine Screening	Multiple partners	1	1	N	84	130/70	0	N	N	0	0	0
41	FM	24	Housewife	Married	Primipara with HIV positive status	Husband positive	0	0	N	88	120/80	1	N	N	0	0	0
42	M	32	Manual Labor	Married	Routine Screening	Multiple partners	1	1	N	78	120/70	0	N	Xerosis	0	0	0
43	M	39	Farmer	Married	Fever, cough with expectoration, vomiting		1	0	N	138	SBP 74 mn	1	Clubbing	Xerosis	0	1	0
44	M	22	Carpenter	Single	Routine Screening	Multiple partners	1	1	N	70	110/80	0	N	N	0	0	0
45	FM	28	Housewife	Married	Fever, cough, wieght loss	Husband positive	0	0	N	110	110/90	1	Platynychia	Xerosis	0	0	0
46	M	41	Business	Married	Ingiunoscrotal swelling, Routine screening	Multiple partners	0	1	N	80	110/80	0	N	N	0	0	0
47	M	50		Married	Opcompound consumption, Routine screening		1	1	N	120	130/90	0	Clubbing	N	0	0	0
48	M	35		Married	Fever, cough with expectoration, altered sensorium	Wife died of HIV	0	1	Febrile	136	110/80	0	N	N	0	0	0
49	M	61	Driver, Ex-serviceman	Married	Fever, vomiting, syncopal attack	Multiple partners	0	1	N	68	80/60	0	Clubbing	N	0	0	0
50	FM	63	Housewife	Married	Routine Screening	Husband positive	0	0	N	74	110/74	0	N	N	0	0	0

RS	CVS	Abdomen Examinatio	CNS	Hb%	TLC	DC			TLyC	Platelet Count	ESR	Peripheral Smear
N	N	Tenderness + in Rt limbar region, no organomegaly, BS+	N	14.3	6300	70	28	2	1764	224000	100	N
N	N	N	N	13.9	6900	46	43	3	2967	140000	30	Mild thrombocytopenia
B/L rhonchi & crepitations	N	N	N	12.3	4600	58	30	12	1380	239000	5	Relative eosinophilia
N	N	N	N	11	7500	58	39	3	2925	228000	60	N
N	N	Tenderness & guarding in epigas. + Rt. Hypochon	N		5118.58				844			
B/L Crepitations + harsh vesicular B/S	N	Tenderness in epigas. + Rt. Hypochon, Hepatomegaly	N	9.4	5400	89	8	3	432	229000	120	Mild NN anemia
N	N	Erythematous rash - Herpes	N	16	6900	56	42	2	2898	153000		
B/L crepitations	N	N	Altered sensorium, Terminal neck rigidity	10.9	5500	73	25	2	1375	127000	25	
B/L ↓B/S with crepitations	N	N	N	9.1	5500	78	19	3	1045	213000	25	NN Anemia
N	N	N	N	9	4300	90	10		430	109000	80	NN Anemia, relative neutrophilia, thrombocytopenia
Stony dull to percuss, Absent B/S on the left	N	N	Altered sensorium (restless, irritable)	9.9	8000	90	10		800	172000	124	NN Anemia with relative neutrophilia
N	N	Splenomegaly	N	11	10900	86	14		1526	381000	48	NN with neutrophilic leucocytosis
N	N	Tenderness in hypogastrium, rigidity, Hepatomegaly	N	9.8	9000	90	7	3	630	120000	45	NN Anemia, relative neutrophilia, thrombocytopenia
N	N	N	N	12.7	15300	90	10		1530	318000	48	NN with neutrophilic leucocytosis
N	N	N	N	7.8	25200	92	5	3	1260	54000	10	Micro. Hypo. Anemia, neutrophilic leucoytosis, Thrombocytopenia
Impaired note with ↓B/S on the left	N	Hepatosplenomegaly	N	9.3	16000	76	23	1	3680	50000	65	NN Anemia, relative neutrophilia, thrombocytopenia
B/L dull note with absent B/S basally	N	distension with shifting dullness	N	7.3	2800	74	24	2	672	214000	130	N
N	N	N	N	11.8	7700	72	25	3	1925	337000	80	N
B/L ↓B/S with crepitations and rhonchi	N	N	N	7.8	8000	86	10		800	158000	105	NN Anemia with monocytosis
N	N	N	N	14.4	6400	70	23	7	1472	311000		N
N	N	N	N	15.9	13000	90	10		1300	293000		
N	N	N	Altered sensorium, Terminal neck rigidity	10.4	9600	81	17	2	1632	279000	100	NN with neutrophilic leucocytosis
N	N	N	N	9	8350	50	48	2	4008		42	
Stony dullness with absent B/S over L lower lung fields	N	N	N	12.2	16700	80	19	1	3173	683000	70	NN, relative neutrophilia, thrombocytosis
N	N	N	N	11.4	8500	58	35	6	2975	435000		
N	N	N	N	8.3	6800	49	34	1	2312	130000	37	
N	N	Tenderness in Rt hypochondrium, hepatomegaly	N	9.6	11200	85	15		1680	118000	100	Micr. Hypo. Anemia with neutrophilia, thrombocytopenia
N	N	Tenderness in epigastrium	N	18.3	10400	90	8	2	832	112000	15	NN with neutrophilic leucocytosis, thrombocytopenia
N	N	N	N	12.2	8500	57	22	21	1870	282000	65	NN with eosinophilia
N	N	Tenderness + in RIF & Renal angle	N	13.2	10500	74	23	3	2415	293000	25	N
N	N	N	N	13.9	5700	58	32	9	1824	168000		NN
N	N	N	N	12.6	8800	56	40	2	3520	191000		NN
N	N	N	N	13.5	9600	69	27	3	2592	203000		NN
↓BS with rhonchi & crepitations on Rt MA, IAA, InterSA	N	N	N	13.4	5200	66	32	1	1664	160000	14	NN
B/L ↓ BS with crepitations	S1, S2 +. Lou	Tenderness + in Rt hypochondrium	N	17	15400	88	12		1848	267000	25	NN with neutrophilic leukocytosis
N	N	N	N	15.9	9400	85	8	2	752	195000	30	NN
B/L ↓ BS with coarse crepitations	N	N	N	10.2	8500	63	26	11	2210	138000	60	NN with eosinophilia
N	N	N	N	14.7	5900	74	25	1	1475	204000	35	NN
N	N	N	N	9.2	7900	88	12		948	131000	50	NN with mild thrombocytopenia
N	N	N	N	10.6	9000	64	35	1	3150		40	
N	N	N	N	12.4	21000	90	10		2100	422000		
N	N	N	N	16.9	6400	42	32	26	2048	144000	48	NN with eosinophilia
B/L ↓B/S with diffuse crepitations	S1, S2+. Tach	N	N	15.7	18029.28				1328	156000		
N	N	N	N	14.8	12300	84	15	1	1845	252000	13	NN with neutrophilic leukocytosis
B/L NVBS with crepitations	N	N	N	10.2	11378.1		9.93		1130	425000		
N	N	N	N	10.7	6700	64	18	18	1206	337000	50	NN with eosinophilia
N	N	N	N	16.4	21000	70	23	2	4830	217000	20	NN with neutrophilic leukocytosis
B/L ↓B/S with crepitations & rhonchi	N	N	Pt disoriented & restless	10	4100	74	22	4	902	277000	95	NN
N	N	N	N	9.7	6700	93	4	1	268	106000	110	NN anemia with thrombocytopenia
N	N	N	N	8.4	3900	48	50	2	1950	130000	90	Pancytopenia

CX-Ray	USG Abdomen	CSF Analysis	B.Urea	S.Creatinine	LFT	Blood Culture	Sputum AFB
↑ bronchovascular marking				1			0
N			45	0.66			0
↑ bronchovascular marking			24	1.1			
	Cholelithiasis + GB sludge, X hypoechoic lesions		30	1.1	N		
B/L non-homogenous opacity in LL							
			16	1.1			
			81	4.8			
Non-homogenous opacity in Rt.mid & lower zone			72	0.53	↓A/G (0.54)		
NRA			26	0.9			
L sided effusion	Hepatomegaly with fatty changes, ascites	P(160), S(18), 0-2 cells (lymphocytes)	60	2.6	SGOT (92), SGPT (30), ALP (821), GGT (309), ↓A/G (0.37)		
N			26	1			
B/L apical lobe infiltrate & consolidation	? Ovarian mass, thickened bowel loops in r lumbar & iliac region		25	0.9	STB(3.1, Indirect), SGOT(130), SGPT(30), ALP(551), GGT(8), ↓A/G (1)		Positive
N			90	2.9			
N			98	3.1	STB(3.4), SDB(1.1), SGOT(108), SGPT(84), ALP(448), GGT(123), ↓A/G(1)		
	Diffuse cystitis, hepatomegaly with fatty changes		77	1.9	STB(3.1), SDB(0.81), SGOT(136), SGPT(218), ALP(306), GGT(163), ↓A/G(0.75)	ESBL +	0
B/L effusion							0
N			29	0.95	N		
B/L non homogenous opacities			22	0.7			0
N							
			33	1.1			
N		Cryptococcal meningitis	21	0.77			
			19	0.5			
L sided pleural effusion	Pseudocyst pancreas		34	1	↓A/G (0.62)		
N	minimal POD collection				N		
N			30	0.8	N		
N	Pelvic appendix with probe tenderness +		18	1.2			
			15	0.79	N		
Rt. Middle lobe opacity			93	1.9			Negative
B/L homogenous opacity			53	1.1			
N			26	1.4			
Non-homogenous opacity in B/L lower zone, hilar congestion, ↑Bronchovascular marking			35	1.5			
			20	0.88			
N	Bulky testis with scrotal collection & internal echoes		42	1.5	↓A/G (0.8)		
N			22				Negative
							Negative
Non-homogenous opacity in L lower zone, obliteration of Cp angle, ↑Bronchovascular marking			62	2.6	N		
	N						
N			29	0.66			Negative
N	Inguinal hernia with omentocoele, mild hepatomegaly						
			26	0.8			
Non-homogenous opacity all over lung fields							
↑Bronchovascular marking	Hepatomegaly		82	2.4			

Sputum G Stain	Sputum C/S	HBsAg	VDRL	CD4+	CD8+	CD3	Urine Routine	Urine Microscopy	Urine C/S	Stool Routine	Stool Microscopy	Cytology	CT Brain						
G +ve cocci +				108		1027	N	N											
				906		2480	N	N											
		0		98		992	N	N											
				307		1895													
		0		110	477.83	610.66													
				44.28	271.78	339.22	N	N											
				125			N	N											
				94.75	217.73	328.06	Trace albumin	UTI, Hematuria											
		1		38			Trace albumin	N											
				18		157													
				45		313	N	N											
				249		1055						FNAC LN - Granulomatous lymphadenitis	? Cerebral abscess - L fronto-temporal						
		0		194						N	N								
				138		1003													
		0		981															
			NR	181			Albumin +	Insignificant bacteriuria											
				78															
		0	Reactive	384															
			NR	61		714													
				624															
				197															
				55.86	169.95	242.15													
			NR	125		560													
				588		1950	Trace albumin	UTI											
			NR	419.9	738.21	1225.26													
		0	NR	160															
				1194	1734.3	127.76	Trace albumin	Insignificant bacteriuria											
				23.5	132.49	172.67													
				172.3	1245.5	1456.66													
		0		277.8	441.34	817.4													
				246		1068													
		0	NR	365		1901	nil												
		0	NR	934	2415														
G-ve & G+ve bacilli		0		672		1865	Trace albumin	Insignificant bacteriuria											
		0		52.45	1034.68	1115.06													
				1591	1075.21														
		1		106.3	915.14	1100.41													
		0		105.9	1399.44	1605.49													
				548	1489		Trace albumin	UTI											
				124															
				280		1024													
				173.4	1381.05	1580.97													
				122.6	122.95	277.57	Trace albumin	UTI											
		0		412.8	8.33	979.51	nil	UTI											
		0		19.07	701.59	750.3													
		0		148.2	1461.77	1753													
				288.1	811.26	1227.79	Trace albumin	Insignificant bacteriuria											
				26									Hypodensity in Rt. Capsuloganglionic region, Calcified area in the L thalamus, Hypodensity in the Post. Limb of Inter						
				34.74	365.78	442.75	Trace albumin	UTI											
				405.3	1719.59	2230.8													