Epidemiological Study on HIV and AIDS Affected Population in North India

THESIS

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by

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Under the Supervision of

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CERTIFICATE

This is to certify that the thesis entitled "**Epidemiological Study on HIV and AIDS Affected Population in North India**" which is submitted by Mr. **Ajay Kumar Sharma**, I.D. No.- **2005PHXF408** for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

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DEDICATION

This project is dedicated to the everlasting Zeal, Enthusiasm, and Perseverance of People living with HIV & AIDS, who despite many odds against them are marching ahead, with their heads high in this Journey of Life. God Bless them all …

AKS…

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Ajay Kumar Sharma

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AIDS	Acquired Immune Deficiency Syndrome
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMOVA	Analysis of Molecular Variance
ANC	Antenatal Clinic
ARC	AIDS Related Complex
ART	Anti retroviral Therapy
ARV	Antiretroviral
AST	Aspartate Aminotransferase
Bil-D	Bilirubin-Direct
Bil-T	Bilirubin-Total
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
bp	Base Pair
CD4+ T	CD4+ T Lymphocyte cells
CD8 T	CD8 T Lymphocyte cells
CDC	Centers for Disease Control and Prevention
Creat	Serum Creatinine
CRF	Circulating Recombinant Form
CTL	Cytotoxic T Lymphocyte
CYP	Cytochrome P-450
°C	Degree Centigrade
dNTP	Deoxy Nucleotide Triphosphate
EDTA	Ethylenediaminetetraacetate
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immunosorbent Assay
EtBr	Ethidium Bromide
FSW	Female Sex Worker
GLDH	Glutamate Dehydrogenase

Glu	Blood Glucose
Gm	Gram
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HIVAN	Human Immunodeficiency Virus Associated Nephropathy
HLA	Human Leukocyte Antigen
HMA	Heteroduplex Mobility Assay
ICMR	Indian Council of Medical Research
IDU	Injecting Drug User
IEC	Institutional Ethical Committee
IFCC	International Federation of Clinical Chemistry
IL	Interleukin
INR	International Normalized Ratio
IRB	Institutional Research Board
IU	International Unit
Kb	Kilo Base Pair
KCL	Potassium Chloride
KFT	Kidney Function Test
L	Liter
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LEE	Liver enzymes elevation
LFT	Liver Function Test
LMP	Low Melting Point
LTR	Long Terminal Repeat
M	Molar
MDH	Malate Dehydrogenase
MELD	Model for End-Stage Liver Disease
$MgCl_2$	Magnesium Chloride

MIET	Meerut Institute of Engineering and Technology
mmol	Milli Molar
MS	Mean square
MSM	Men Who Have Sex with Men
NaCl	Sodium Chloride
NACO	National AIDS Control Organization
NACP	National AIDS Control Program
NADH	Nicotinamide adenine dinucleotide (reduced)
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
NGO	Non Governmental Organization
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NTSYS	Numerical Taxonomy System
OD	Optical Density
OI	Opportunistic Infection
OTU	Operational Taxonomic Unit
PBMC	Peripheral Blood Mononuclear Cells
PCP	Pneumocystis pneumonia
PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
PIC	Polymorphic Information Content
PJB	Punjab
PLHA	People Living with HIV/AIDS
PSAT	Primer Specific Annealing Temperature
psi	Pounds Per Square Inch
QA	Quality Assurance
QC	Quality Control
RAJ	Rajasthan
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
Rp	Resolving Power

RT	Room Temperature
SDS	Sodium Do-Decyl Sulphate
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SIV	Simian Immunodeficiency Virus
SNP	Single Nucleotide Polymorphism
SS	Sum of Squares
STD	Sexually Transmitted Disease
TB	*Bacillus tubercle* (Tuberculosis)
TBE	Tris Borate EDTA
TC	Total Cholesterol
TE	Tris EDTA
TG	Triglycerides
TLC	Total Lymphocyte Count
Tris-HCL	Tris(hydroxymethyl)aminomethane Hydochloride
UNAIDS	United Nations Programme on HIV and AIDS
UPGMA	Un-weighted Pair Group Method with Arithmetic Mean
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

CHAPTER – 1

INTRODUCTION

CHAPTER 1.0

Introduction

The first recorded sample of human immunodeficiency virus (HIV) was discovered in 1959 in a blood specimen obtained at Leopoldville (now Kinshasa) in the Belgian Congo [1]. This was the first known death caused by acquired immune deficiency syndrome (AIDS). In the early 1980s, the first recognized cases of the AIDS occurred among homosexual men in the United States. These men suddenly began to develop rare opportunistic infections and cancers that seemed stubbornly resistant to any treatment. At this time, AIDS did not yet have a name, but it quickly became obvious that all the men were suffering from a common syndrome [2]. By 1983, the etiological agent HIV was identified.

The virus is thought to have originally affected chimpanzees. Scientists believe that the chimpanzee version of the immunodeficiency virus (called simian immunodeficiency virus or SIV) most likely was transmitted to humans and mutated into HIV when humans hunted these chimpanzees for meat and came into contact with their infected blood. It is now established that HIV causes AIDS. By the mid-1980's, it became clear that the virus had spread, largely unnoticed, around the world. Since then, the global AIDS epidemic has become one of the greatest threats to human health and development. At the same time, much has been learnt about the science of AIDS, as well as how to prevent and treat the disease.

AIDS is caused by the retrovirus, HIV and characterized by profound immunosuppression that leads to opportunistic infections, secondary neoplasms, and neurologic manifestations. HIV basically provokes an infection, which destroys the body`s immune system and AIDS is the advanced stage of this syndrome, when the immune system becomes irreparably damaged, engendering multiple infections and cancers. AIDS include a number of unusual and severe infections, cancers and debilitating illnesses, resulting in severe weight loss or wasting away, and diseases affecting the brain and central nervous system.

Over decades, the virus slowly spread across Africa and later into other parts of the world.

1.1 Description of AIDS and HIV Infection

The immune system is a network of cells, organs and proteins that work together to defend and protect the body from potentially harmful, infectious microorganisms (microscopic life-forms), such as bacteria, viruses, parasites and fungi. The immune system also plays a critical role in preventing the development and spread of many types of cancer. When the immune system is missing one or more of its components, the result is an immunodeficiency disorder such as AIDS. Lymphocytes (white blood cells) are one of the main types of immune cells that make up the immune system. There are two types of lymphocytes: B cells and T cells (two main subtypes include; helper T cells with CD4 marker proteins and cytotoxic T cells with CD8 marker proteins). B cells secrete antibodies (proteins) into the body's fluids to ambush and attack antigens (foreign proteins such as bacteria, viruses or fungi) while T cells directly attack and destroy infected or malignant cells in the body.

The $CD4^+$ T lymphocyte is the primary target for HIV infection because of the affinity of the virus for the CD4 surface marker. When HIV gains entry into the body, the virus is too strong against the body's immune power (helper T cells and cytotoxic T cells) and thereby invades these cells and starts to reproduce itself, eventually not only killing the CD4 T cells, but also spreading to infect neighboring healthy cells [3]. Although HIV targets T-cells and other cells in the body, it thrives mainly in the lymph nodes—another important part of the immune system. Each lymph node has a netlike structure inside it that acts as a protective filter by trapping virus and infected T-cells. But as healthy T-cells move through contaminated lymph nodes, they are infected by HIV. Particularly during the early stage of the disease, lymph nodes contain more infected cells than the blood. The viral load, or amount of virus in circulation, is a barometer of disease progression.

1.2 Diversity of HIV

The HIV epidemic unfolded in India in 1986. India is experiencing a rapid and extensive spread. HIV prevalence is higher than 1% in Andhra Pradesh, Karnataka, Maharashtra, Manipur, Nagaland and Tamil Nadu. Multiple HIV epidemics are being observed among certain population groups in various parts of this vast country. The

genomic diversity of human immunodeficiency virus type-I (HIV-1) in India is well elucidated [4]. Phylogenetic analyses, based on the nucleotide or deduced amino acid sequences of *env*, *gag* and *pol* genes of numerous HIV-1 strains have revealed three distinct clades of viruses, which have been termed as groups M (main), N (new or non-M, non-O) and O (outlier). Molecular epidemiology of HIV-1 strains circulating in different regions of India was carried out [5]. These studies concluded that HIV-1 subtype C was predominant in India accounting for 85-90 % of all isolates. Other subtypes observed included subtypes A, Thai B, E and putative recombinants. Further, as the epidemic evolves, subtype C strains are likely to replace subtype Thai B in the Northeast. In 1986, a second type of HIV, called HIV-2, was isolated from AIDS patients in West Africa [6, 7]. HIV-2 has the same modes of transmission as HIV-1 with similar opportunistic infections and AIDS. In persons infected with HIV-2, immunodeficiency seems to develop more slowly and to be milder, and those with HIV-2 are comparatively less infectious early in the course of infection. As the disease advances, HIV-2 infectiousness seems to increase; however, compared with HIV-1, the duration of this increased infectiousness is shorter. HIV-2 infections are predominantly found in Africa [8].

1.3 Symptoms of AIDS and HIV Infection

Immediately following infection with HIV, most individuals develop a brief, nonspecific “viral illness” consisting of low grade fever, rash, muscle aches, headache and/or fatigue. Like any other viral illness, these symptoms resolve over a period of five to ten days. People infected with HIV may have no symptoms for ten years or longer, but they can still transmit the infection to others during this symptom-free period [9]. Acute HIV infection progresses initially as asymptomatic HIV infection and later to early symptomatic HIV infection. However, their T cells become progressively destroyed or inactivated by the virus as the other viruses, parasites or cancer cells (agents for opportunistic infections) overwhelms the depleted body's defense and can multiply within the body without fear of destruction. When this destruction has progressed to a critical point, symptoms of AIDS [10, 11] appear as follows:

- extreme fatigue
- rapid weight loss from an unknown cause (more than 4.5 Kilo grams in two months for no reason)
- appearance of swollen or tender glands in the neck, armpits or groin, for no apparent reason, lasting for more than four weeks
- unexplained shortness of breath, frequently accompanied by a dry cough, not due to allergies or smoking
- persistent diarrhea
- intermittent high fever or soaking night sweats of unknown origin
- a marked change in an illness pattern, either in frequency, severity, or length of sickness
- appearance of one or more purple spots on the surface of the skin, inside the mouth, anus or nasal passages
- whitish coating on the tongue, throat or vagina
- forgetfulness, confusion and other signs of mental deterioration

Most individuals infected with HIV, if not treated, will develop AIDS. There is a small group of patients who develop AIDS very slowly, or never at all. These patients are called non-progressors, and many seem to have a genetic difference that prevents the virus from attaching to certain immune receptors [12]. The progression of the HIV infection from primary infection to "full-blown" AIDS can take as short as a year to as long as 10 to 20 years [13]. According to the Center for Disease Control and Prevention (CDC) classification of 1993 [14], a person is considered to be affected with AIDS when its $CD4^+$ T lymphocytes are less than 200/µL, or they have an AIDS-defining condition. The AIDS-defining conditions are:

- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)

- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma (primary), of brain
- Mycobacterium avium complex or *M. kansasii*, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV

In addition to the $CD4^+$ count, HIV RNA load, and basic screening laboratory tests, regular vaginal Pap smears are important to monitor HIV infection, due to the increased risk of cervical cancer in immuno-compromised patients [15]. Anal Pap smears to detect potential cancers may also be important in both HIV infected men and women. Common bacteria, yeast, parasites, and viruses that ordinarily do not cause serious disease in people with healthy immune systems can cause fatal illnesses in people with AIDS [16]. The symptoms of AIDS are primarily the result of infections that do not normally develop in individuals with healthy immune systems. These are called opportunistic infections. Patients with AIDS have had their immune system depleted by HIV and are very susceptible to such opportunistic infections.

Common AIDS-related opportunistic infections are caused by fungi like Candida (topical and systemic yeast infections), bacteria like tuberculosis, and viruses like hepatitis and herpes.

People who are not infected with HIV may also develop these diseases; the presence of any one of these conditions does not mean the person has AIDS. To be diagnosed with AIDS, a person must be infected with HIV. Some people infected with HIV may develop a disease that is less serious than AIDS, referred to as AIDS Related Complex (ARC). ARC is a condition caused by the AIDS virus in which the patient tests positive for HIV infection and has a specific set of clinical symptoms [17]. However, the symptoms in ARC patients are often less severe than those with classic AIDS because the degree of destruction of the immune system has not progressed as far as it has in patients with classic AIDS. Symptoms of ARC may include loss of appetite, weight loss, fever, night sweats, skin rashes, diarrhea, tiredness, lack of resistance to infection or swollen lymph nodes.

1.4 Modes of Transmission [18]

HIV is transmitted primarily by sex (anal, vaginal or oral sex with an infected partner), by injections (sharing contaminated needles for drug use or accidental piercing with a contaminated needle), infected blood transfusion, infected cutting tools (skin-piercing instruments, tattoo needles, circumcision instruments) or from infected mother to child (during pregnancy, delivery, and breastfeeding) [19]. Infected semen and vaginal fluids, infected blood and blood products lead to the transmission of HIV. Drug abuse with unsterilized needles is another high-risk activity. Unprotected sex with multiple partners is the primary cause of infection. During unprotected sex, the infected fluid could enter the bloodstream through a tiny cut or a sore. Anal penetration has a higher risk of transmission, which is why a high percentage of homosexuals develop the disease. Bleeding during sex also raises the chances of infection, particularly unprotected sex during menstrual periods and anal intercourse. Although traces of HIV have been detected in body fluids (saliva, urine, feces and tears) there is no evidence that HIV spreads through these fluids. HIV is not water-borne, air-borne or transmitted through mosquitoes and other insects.

Many myths exist about how HIV is transmitted, many of which are culturally specific. It is clearly indicated through scientific studies that HIV is not transmitted through casual contact or social interaction – rather transmission is linked to intimate contact.

HIV is not transmitted by:

- Kissing, hugging, handshaking
- Sneezing, coughing, sharing glasses/utensils, etc.
- Injections or surgery with sterile needles and tools
- Safer sex using condoms
- Tears, sweat, saliva, vomit, feces or urine
- Using toilets, drinking fountains, public swimming pools
- Insect bites
- Working, socializing or living with a person with HIV

1.5 Prevention from AIDS and HIV Infection [20]

The only way to protect from contracting AIDS sexually is to abstain from sex outside of a mutually faithful relationship with a partner whom the person knows is not infected with the AIDS virus. Otherwise, risks can be minimized if they:

- Don't have sexual contact with anyone who has symptoms of AIDS or who is a member of a high risk group for AIDS.
- Avoid sexual contact with anyone who has had sex with people at risk of getting AIDS.
- Don't have sex with prostitutes.
- Avoid having sex with anyone who has multiple and/or anonymous sexual partners.
- Avoid oral, genital and anal contact with partner's blood, semen, vaginal secretions, feces or urine. Unless they know with absolute certainty that their partner is not infected, a latex condom should be used during each sexual act, from start to finish. The use of a spermicidal agent may provide additional protection.
- Avoid anal intercourse altogether.

- Don't share toothbrushes, razors or other implements that could become contaminated with the blood of anyone who is or might be infected with the AIDS virus.
- Exercise caution regarding procedures, such as acupuncture, tattooing, and ear piercing, etc., in which needles or other non-sterile instruments may be used repeatedly to pierce the skin and/or mucous membranes. Such procedures are safe if proper sterilization methods are employed or disposable needles are used. Asking the technical staff about precautions to be taken before undergoing such procedures.
- If an individual is scheduling surgery in the near future, and is able, they could consider donating blood for their own use. This will eliminate completely the already very small risk of contracting AIDS through a blood transfusion. It will also eliminate the risk of contracting other blood-borne diseases (such as hepatitis) from a transfusion.

Some people apparently remain well after infection of the AIDS virus. They may have no physically apparent symptoms of illness. However, if proper precautions are not used with sexual contacts and/or intravenous drug use, these infected individuals can spread the virus to others.

1.6 Treatment of AIDS and HIV Infection

There is no cure for AIDS at this time. However, a variety of treatments are available that can delay the progression of disease for many years, and improve the quality of life of those who have developed symptoms. Antiretroviral therapy suppresses the replication of the HIV virus in the body. A combination of several antiretroviral agents, termed highly active antiretroviral therapy (HAART), has been highly effective in reducing the number of HIV particles in the blood stream, as measured by a blood test called the viral load. This can help the immune system recover from the HIV infection and improve T-cell counts. With consistent and timely treatment [21], it is possible that individuals infected with HIV will never exhibit AIDS symptoms and decreases the potential for HIV to be transmitted sexually. HAART can significantly improve health related quality of life [22].

Anti-HIV medications [23] fall into four classes:

1. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs), such as nevirappine (Viramune) and efavirenz (Sustiva), bind to and block the action of reverse transcriptase, a protein that HIV needs to reproduce.

2. Nucleoside Reverse Transcriptase Inhibitors (NRTIs), such as zidovudine (Retrovir), tenofovir DF (Viread), and stavudine (Zerit), are faulty versions of building blocks that HIV needs to make more copies of it. When HIV uses an NRTI instead of a normal building block, reproduction of the virus is stalled.

3. Protease Inhibitors (PIs), such as lopinavir/ritonavir (Kaletra), disable protease, a protein that HIV needs reproduce itself.

4. Fusion Inhibitors, such as enfuvirtide (Fuzeon), are newer treatments that work by blocking HIV entry into cells.

Although not a cure for HIV, these treatments have been enormously effective for the past several years. There is good evidence that if the levels of HIV remain suppressed and the $CD4^+$ count remains high (above 200), life can be significantly prolonged and improved [24, 25]. The EuroSIDA study, comparing the early HAART period to pre-HAART and later HAART (1998 to 2002) treatment periods, found a sustained decrease in mortality and progression to AIDS with ongoing HAART [26]. However, HIV may become resistant to HAART in patients who do not take their medications on schedule every day. Treatment with HAART is not without complications. HAART is a collection of different medications, each with its own side effects. Some common side effects are nausea, headache, weakness, malaise, and fat accumulation on the back and abdomen ("buffalo hump"). When used long-term, these medications increase the risk of heart attack by affecting fat breakdown, specifically through increasing lipids and glucose levels. The goal is to get the $CD4^+$ count as close to normal as possible, and to suppress the HIV viral load to an undetectable level.

1.7 Epidemiology

According to the Joint United Nations Programme on HIV and AIDS (UNAIDS), an estimated 34 million people [31.6 million–35.2 million] were living with HIV worldwide, at the end of 2010, which is up by 17% from 2001 estimates. The total number of people living with HIV/AIDS (PLHA) in India is estimated at 24 lakh (19.3 – 30.4) in 2009 [27]. Children (<15 years) account for 3.5% of all infections, while 83% are the in age group 15-49 years. Of all HIV infections, 39% (9.3 lakh) are among women. The four high prevalence states of South India (Andhra Pradesh – 5 lakh, Maharashtra – 4.2 lakh, Karnataka – 2.5 lakh, Tamil Nadu – 1.5 lakh) account for 55% of all HIV infections in the country. The states of Punjab, Orissa, Rajasthan & Madhya Pradesh have 50,000 – 1 lakh HIV infections each and together account for 12% of HIV infections. These states, in spite of low HIV prevalence, have large number of PLHA due to the large population size. Of the 1.2 lakh estimated new infections in 2009, the six high prevalence states account for only 39% of the cases, while the states of Orissa, Bihar, West Bengal, Uttar Pradesh, Rajasthan, Madhya Pradesh and Gujarat account for 41% of new infections. The estimated adult HIV prevalence in India was 0.32% (0.26% – 0.41%) in 2008 and 0.31% (0.25% – 0.39%) in 2009. The adult prevalence is 0.26% among women and 0.38% among men in 2008, and 0.25% among women and 0.36% among men in 2009. The states of Goa, Chandigarh, Gujarat, Punjab and Tamil Nadu have shown estimated adult HIV prevalence greater than national prevalence (0.31%).

1.8 National Response

The Ministry of Health and Family welfare (Government of India) established a National AIDS Control Program (NACP) unit shortly after the reporting of first AIDS case in 1986. Initially, the program's principal activity was limited to monitoring HIV infection rates among risk populations in select urban areas. In 1991, the strategy was revised to focus on blood safety, prevention among high-risk populations, raising awareness in the general population, and improving surveillance. A semi-autonomous body, the National AIDS Control Organization (NACO), was established under the Ministry of Health and Family Welfare to implement this program. This "first phase" of the NACP lasted from 1992 -1999. Subsequently, the

second phase of NACP began in 1999 and run till March, 2006. Under this phase, greater emphasis has been placed on targeted interventions for high-risk groups, preventive interventions among the general population, and involvement of NGOs and other sectors and line departments, such as education, transport and police. Phase III (NACP III) was launched in July 2007 with the goal of Halting and Reversing the Epidemic by the end of project period in mid 2012. The NACO has now initiated the process to start the next phase of the program (NACP-IV) to ensure completion of the reversal of the epidemic through enhanced prevention linked with care support and treatment. In brief, while the government's response has scaled up markedly over the last decade, major challenges remain in raising the overall effectiveness of state-level programs, expanding the participation of other sectors, and increasing safe behavior and reducing stigma associated with HIV +ve people among the population.

There are numerous non-governmental organizations (NGOs) working on HIV/AIDS issues in India at the local, state, and national levels. Projects include targeted interventions with high risk groups, direct care of people living with HIV/AIDS, general awareness campaigns and care for AIDS orphans. Funding for NGOs comes from a variety of sources: the federal or state governments of India, international donors, and local contributions.

For the present study a total of 319 human blood samples were obtained from respective medical centres at Ludhiana (Punjab; State-1) and Jodhpur (Rajasthan; State-2) between September, 2010 to June, 2011, with due permission from the concerned authorities. Out of these, 148 blood samples were HIV +ve, 53 AIDS affected and 118 blood samples from age and sex matched normal individuals. This study was conducted at Department of Biotechnology, Meerut Institute of Engineering and Technology (MIET), Meerut. Due permission was taken from Institutional Research Board (IRB) and Institutional Ethical Committee (IEC) of MIET for conducting the present project. The blood samples were analyzed for various biochemical tests (Liver function tests-LFTs, Kidney function tests-KFTs, Lipid function tests and test for blood sugar) in an authorized medical diagnostic laboratory.

To complement our biochemical analysis, we also performed molecular marker studies with the help of four different sets of molecular primers. Out of which one set (4 primers) were selected from one of the reviewed paper [28] on the basis of amplified product size and rest three sets were designed through National Center for Biotechnology Information (NCBI) Primer basic local alignment search tool (BLAST) [29]. For molecular analysis, we have selected twenty two samples each from two different geographical regions (Punjab and Rajasthan) and undertook polymerase chain reaction (PCR) analysis, which was based on random gene amplification. This data was further analyzed by Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) [30] based Jacquard's similarity coefficient. This was done to find out similarity or differences between samples from two geographical regions. The same data was also subjected to analysis of molecular variance (AMOVA) for estimating population differentiation.

1.9 Objectives

The present study was carried out with the following objectives:

- To establish the correlation between HIV infection and various metabolic dysfunction tests (Liver Function Tests (LFTs), Kidney Function Tests (KFTs), Lipid function tests and test for blood sugar).
- To develop relationship between $CD4^+$ cell count and various biochemical parameters.
- To examine phylogenetic relationships amongst HIV infected population of North India and to generate reference data of molecular primers for analysis of HIV +ve subjects.
- To find out correlation between biochemical (LFTs) and molecular analysis of HIV +ve individuals

CHAPTER – 2

REVIEW OF LITERATURE

CHAPTER 2.0

Review of Literature

A major obstacle facing many lower income countries in establishing and maintaining (human immunodeficiency virus) HIV treatment programmes is the scarcity of trained health care manpower. To address this problem, the World Health Organization (WHO) has recommend task shifting to HIV-infected peers. A model of HIV care was designed by Kaloustian et al. [31] in a western Kenya study that utilize HIV-infected patients, community care coordinators, to care for their clinically stable peers with the assistance of pre-programmed personal digital assistants. In this model, instead of monthly clinic visits, the patients were seen every three months in clinics and monthly by their community care coordinators at community centre during the interim two months. An analysis of the continuous feedback and quality improvement programme was used to assess this model. It was found that an antiretroviral treatment (ART) delivery model shifting patient monitoring and antiretroviral dispensing tasks into the community by HIV-infected patients was both acceptable and feasible.

2.1 Anti retroviral Therapy (ART)

Kumarasamy et al. [32] reviewed the changing natural history of HIV disease before and after the introduction of generic antiretroviral therapy (ART) in southern India. The number of individuals seeking treatment for infection with HIV increased as the cost of highly active antiretroviral therapy (HAART) decreased 20-fold after the introduction of generic HAART in India in the year 2000. The incidence of tuberculosis and opportunistic infections decreased to 2 cases per 100 person-years.

Easterbrook et al. [33] compared the rate of immunological progression prior to ART and the virological response to ART in patients infected with subtype B and four non-B HIV-1 subtypes (A, C, D and the circulating recombinant form, CRF02-AG) in an ethnically diverse population of HIV-1-infected patients in south London. A random sample of 861 HIV-1-infected patients attending HIV clinics at King's and St Thomas' hospitals' were subtyped using an in-house enzyme-linked immunoassay and *env* sequencing. No significant differences were reported in rate of $CD4^+$ cell decline, initial response to HAART and subsequent rate of virological rebound for

subtypes B, A, C and CRF02-AG. However, a statistically significant four-fold faster rate of CD4 decline (after adjustment for gender, ethnicity and baseline $CD4^+$ count) was observed for subtype D.

A long-term outcome of ART in children was documented by Isaakidis et al. [34] in a resource-limited setting in Cambodia in which they assess the two-and three-year survival, CD4 evolution and virological response among children on ART. Children treated with first-line ART for at least 24 months were assessed with viral load testing and genotyping. The group reported that good survival, immunological restoration and viral suppression can be sustained after two to three years of ART among children in resource constrained settings and emphasized on increased access to routine virological measurements for timely diagnosis of treatment failure.

Gomber et al. [35] studied the profile of HIV infected children from Delhi and their response to antiretroviral treatment. The clinical features, immunological profile ($CD4^+$ count) and response to ART of 100 children attending the centres between December 2008 to June 2009 were recorded in a structured proforma. Most common clinical presentation was fever (83%), cough (50.8%) and diarrhea (38.9%). Tuberculosis was the most common opportunistic infection seen in 11% of children. 59% of enrolled children were malnourished. ART was initiated in 33 children. Children who were on ART had a significant improvement in both clinical and immunological staging at the 6 months follow up. Immunological response (rise in CD4 count) to ART was better in children with lesser degree of immunosuppression. The measure of agreement between the clinical and immunological stage at presentation was poor. Baseline CD4 counts rather than clinical staging can be a primary determinant for initiation of ART in HIV infected children. Drapalo et al. [36] in their study conclude that long-lasting HAART seems to decrease cystatin C concentration and thus potentially improves renal function.

HIV-1 compartmentalization in diverse leukocyte populations during antiretroviral therapy was studied by Potter et al. [37]. According to their study CD4 T lymphocytes are the primary target of human immunodeficiency virus type-1 (HIV-1), but there is increasing evidence that other immune cells in the blood, including CD8 T lymphocytes and monocytes, are also productively infected. They conducted a

detailed investigation of HIV-1 diversity and genetic structure in CD4 T cells, CD8 T cells, and monocytes of 13 patients receiving HAART. Analysis of molecular variance and non-parametric tests performed on HIV-1 envelope sequences provided statistically significant evidence of viral compartmentalization in different leukocyte populations. Analysis of viral genetic variation in different leukocyte populations demonstrated the action of founder effects as well as significant variation in the extent of genetic differentiation between sub-populations among patients. Compartmentalization of the virus in different leukocytes may have significant implications for current models of HIV-1 population genetics and contribute to the highly variable way in which drug resistance evolves in different individuals during HAART. HAART can reduce HIV-1 viremia to clinically undetectable levels. Despite this dramatic reduction, some virus is present in the blood. In addition, a long-lived latent reservoir for HIV-1 exists in resting memory $CD4^+$ T cells. This reservoir is believed to be a source of the residual viremia and is the focus of eradication efforts.

Brennan et al. [38] analysed the HIV-1 viremia and provirus in resting $CD4^+$ T cells and reveals a novel source of residual viremia in patients on ART. According to their study residual viremia is genetically distinct from proviruses in activated $CD4^+$ T cells, monocytes, and unfractionated peripheral blood mononuclear cells and concluded that some of the residual viremia in patients on HAART stems from an unidentified cellular source other than $CD4^+$ T cells has implications for eradication efforts. The researchers used two measures of population structure—analysis of molecular variance (AMOVA) and the Slatkin-Maddison test—to demonstrate that the residual viremia is genetically distinct from proviruses in resting $CD4^+$ T cells but those proviruses in resting and activated $CD4^+$ T cells belong to a single population.

Drain et al. [39] reviewed the importance of micronutrients in HIV-positive persons receiving HAART. In HIV-infected persons, low serum concentrations of vitamins and minerals, termed micronutrients, are associated with an increased risk of HIV disease progression and mortality. Micronutrient supplements can delay HIV disease progression and reduce mortality in HIV-positive persons not receiving HAART. With the transition to more universal access to HAART, a better understanding of micronutrient deficiencies and the role of micronutrient supplements

in HIV-positive persons receiving HAART has become a priority. Kaiser et al. [40] studied the effect of micronutrient supplementation in increasing the $CD4^+$ count in HIV-infected individuals on HAART in a prospective, double-blinded, placebo-controlled trial. The study examined the effect of micronutrient supplementation on immunologic parameters as the primary end point. Micronutrient supplementation can significantly improve $CD4^+$ cell count reconstitution in HIV-infected patients taking HAART.

Muthuraj et al. [41] found lower serum zinc, calcium and albumin levels in pulmonary tuberculosis patients co-infected with HIV in comparison to normal (control) healthy individual. Hence, the National AIDS Control Organization (NACO) in India is providing nutritional supplements to those HIV-infected cases inducted for ART and nutritional counseling for others as a part of a national policy. Khalili et al. [42] evaluated the nutritional status and serum zinc and selenium levels of Iranian subjects who were newly diagnosed with HIV infection. The group reported that malnutrition is found to be prevalent in Iranian HIV infected individuals and low serum zinc and selenium levels are common in this population.

Miguez et al. [43] in their *in vitro* studies suggest a dose-response relationship with every increase of 50 mg/dl in cholesterol related to a parallel rise of 50 $CD4^+$ cells. Livio et al. [44] concluded that metabolic and anthropometric parameters contribute to ART-mediated $CD4^+$ T cell recovery in HIV-1-infected individuals. The degree of immune reconstitution achieved in response to suppressive ART is associated with baseline individual characteristics, such as pre-treatment $CD4^+$ count, levels of viral replication, cellular activation, choice of treatment regimen and gender. However, the combined effect of these variables on long-term CD4 recovery remains elusive, and no single variable predicts treatment response.

Aranzabal et al. [45] studied the influence of liver fibrosis on HAART–associated hepatotoxicity in patients with HIV and hepatitis C virus (HCV) co-infection. The group reported that HAART-associated hepatotoxicity correlates with liver histological stage in patients co-infected with HIV and HCV. Nishijima et al. [46] examined the impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients in a retrospective cohort study of Japanese

patients. The incidence of tenofovir-associated renal dysfunction, defined as more than 25% decrement of estimated glomerular filtration rate (eGFR) from the baseline, was determined. Tenofovir-related renal dysfunction occurred in 97 (19.6%) patients (incidence: 10.5 per 100 person-years). They concluded that the incidence of tenofovir-associated renal dysfunction in Japanese patients was high. Small body weight was identified as an independent risk factor for tenofovir-associated renal dysfunction and close monitoring of renal function was advocated for patients with small body weight treated with tenofovir.

2.2 Opportunistic Infections

AIDS-defining opportunistic illnesses are the major cause of morbidity and mortality among persons infected with HIV. As a result of new treatments that reduce mortality for persons with AIDS, the number of persons living with AIDS is increasing, and the incidence of AIDS is decreasing. Kumarasamy et al. [47] reviewed clinical profile of HIV in India. The clinical course of HIV disease and pattern of opportunistic infections varies from patient to patient and from country to country. The clinical profile of HIV disease in India includes a wide range of conditions like tuberculosis, cryptococcal meningitis, popular pruritic eruptions, and cytomegalovirus retinitis, among others. Tuberculosis is the most common opportunistic infection (OI) in Indian patients with HIV. Occurrence of various AIDS associated illnesses determines disease progression. Mean survival time of Indian patients after diagnosis of HIV is 92 months. In this review, they discussed the clinical profile of HIV disease through an organ system-based approach. The group emphasized that with the availability of ART at lower cost, the clinical profile of HIV disease in India is now changing to include drug related toxicities and immune reconstitution syndrome.

In order to document the spectrum and determine the frequency of various OIs and non-infectious opportunistic diseases, Sharma et al. [48] evaluated 135 hospitalised HIV-infected patients from North India. The group reported that fever (71%) and weight losses (65%) were the commonest presenting symptoms. Heterosexual transmission was the commonest mode of HIV-acquisition. Tuberculosis (TB) was the commonest OI (71%) followed by candidiasis (39.3%), Pneumocystis jiroveci pneumonia (PCP) (7.4%), cryptococcal meningitis and cerebral

toxoplasmosis (3.7% each). Most of the cases of TB were disseminated (64%). Apart from other well-recognised OIs, two patients had visceral leishmaniasis. Two cases of HIV-associated lymphoma were encountered. $CD4^+$ cell counts were done in 109 patients. Majority of the patients (82.6%) had $CD4^+$ counts <200 cells/μL. Fifty patients (46%) had $CD4^+$ counts <50 cells/μL. Only 50 patients (37%) received ART. Twenty one patients (16%) died during hospital stay. All but one deaths were due to TB (16 patients; 76%) and PCP (4 patients; 19%). They further reported that a wide spectrum of disease, including both OIs and non-infectious opportunistic diseases, were seen in hospitalised HIV-infected patients from North India. Tuberculosis remains the most common OI and is the commonest cause of death in these patients.

Jones et al. [49] evaluated the surveillance for AIDS-defining opportunistic illnesses from 1992-1997 in U.S. The group reported that the incidence declined significantly for each of 15 of the 26 specific AIDS-defining OIs ($p<0.05$). PCP was the most common AIDS-defining OI to occur first (PCP was the first OI to occur for 36% of HIV-infected persons), the most common incident AIDS-defining OI (274 cases per 1000 person-years), and the most common AIDS-defining OI to have occurred during the course of AIDS (53% of persons who died with AIDS had PCP diagnosed at some time during their course of AIDS). Of persons with $CD4^+$ T-lymphocyte counts <500 cells/μL, the number with prescriptions for triple combination therapy increased from zero in 1992 to 40% in 1997, and 80% of persons had a prescription for any antiretroviral therapy in 1997. Of persons with $CD4^+$ T-lymphocyte counts <200 cells/μL, the percentage with prescriptions for PCP prophylaxis remained stable from 1992 through 1997 (range: 75% to 80%). Of persons with $CD4^+$ T-lymphocyte counts <50 cells/μL, the percentage with prescriptions for MAC prophylaxis increased from 9% in 1992 to 44% in 1997. The incidences of many OIs are decreasing primarily because of advances in HIV-related therapy. However, OIs are still occurring, especially when patients access care late during the course of disease. Even after accessing care, persons may develop OIs because of lack of prescription for prophylaxis, ARV drug resistance, or poor adherence to therapy.

To evaluate the possible role of parasitemia on Chagas' disease reactivation in Chagas' disease/ HIV coinfection cases and the impact of HIV coinfection on *Trypanosoma cruzi* genetic diversity, 71 patients with Chagas' disease (34 HIV +ve and 37 HIV -ve) were surveyed by Ramirez et al. [50] in Brazil. Also 92 *T. cruzi* stocks from 47 chronic chagasic patients (29 HIV +ve and 18 HIV -ve) were isolated and analyzed by multilocus enzyme electrophoresis and a random amplified polymorphic DNA (RAPD) procedure. The group reported no apparent association between given *T. cruzi* genotypes and specific clinical forms of Chagas' disease/HIV associations. Adewole et al. [51] studies Hepatitis B virus (HBV) and HCV co-infection in Nigerian patients with HIV infection in order to determine the seroprevalence of HBV and HCV among HIV infected individuals and its impact on pattern of presentation. They observed that the prevalence of HIV and hepatitis co-infection rises with age except for hepatitis C and suggested that the co-infection with HBV (common among the studied HIV-infected patients) should be a major consideration in the initiation and choice of therapy.

2.3 HIV Infection and AIDS

AIDS has changed over the years as a result of an increasing appreciation of the wide spectrum of clinical manifestations of infection with HIV. Adler [52] described the basics of AIDS in context with the development of the epidemic. As per the researcher, AIDS is defined as an illness characterised by one or more indicator diseases. In the absence of another cause of immune deficiency and without laboratory evidence of HIV infection (if the patient has not been tested or the results are inconclusive), certain diseases when definitively diagnosed are indicative of AIDS. Also, regardless of the presence of other causes of immune deficiency, if there is laboratory evidence of HIV infection, other indicator diseases that require a definitive, or in some cases only a presumptive, diagnosis also constitute a diagnosis of AIDS. In 1993 the Centers for Disease Control and Prevention (CDC) in the United States of America (USA) extended the definition of AIDS to include all persons who are severely immunosuppressed (a CD4 count $< 200 \times 10^6$ cells/l) irrespective of the presence or absence of an indicator disease. For surveillance purposes this definition has not been accepted within the United Kingdom (UK) and Europe. In these

countries AIDS continues to be a clinical diagnosis defined by one or more of the indicator diseases mentioned.

The World Health Organization (WHO) also uses this clinically based definition for surveillance within developed countries. WHO, however, has developed an alternative case definition for use in sub-Saharan Africa. This is based on clinical signs and does not require laboratory confirmation of infection. Subsequently this definition has been modified to include a positive test for HIV antibody. The rapid evolution of HIV has led to the speculation that viral strains currently infecting individuals may be more pathogenic than those infecting patients earlier in the epidemic, resulting in increased rates of HIV disease progression in individuals infected more recently. Any changes to the incubation period are of interest for those studying the natural history of HIV, especially for those involved in predicting the future of the epidemic.

Lepri et al. [53] investigated the temporal changes in the rate of HIV progression and the challenges and limitations involved. The group emphasized on the study whether individuals currently at a certain stage of disease experience faster or slower disease progression than individuals at the same stage of disease some years earlier. This approach should reveal any changes to the incubation period that have occurred as a result of improvements in clinical care.

Carre et al. [54] observed that seroconversion does not occur in relation with incubation period. The group also concluded that early treatment with ARVs and prophylaxis had been beneficial in individuals infected through sexual exposure. Improved rate of progression of AIDS was reported by Enger et al. [55] in individuals followed over a period of time, particularly, those with low CD4 cell counts [56].

The most up-to-date estimates demonstrate very heterogeneous spread of HIV-1, and more than 30 million people are now living with HIV-1 infection, most of them in sub-Saharan Africa. Cohen et al. [57] reviewed the spread, treatment, and prevention of HIV-1 as the evolution of a global pandemic. The efficiency of transmission of HIV-1 depends primarily on the concentration of the virus in the infectious host. Although treatment with antiviral agents has proven a very effective

way to improve the health and survival of infected individuals, as we discuss here, the epidemic will continue to grow unless greatly improved prevention strategies can be developed and implemented. No prophylactic vaccine is on the horizon. The group concluded that several behavioral and structural strategies have made a difference—male circumcision provides substantial protection from sexually transmitted diseases (STDs), including HIV-1, and the application of ARV agents for prevention holds great promise. The HIV-1 pandemic is a complex mix of diverse epidemics within and between countries and regions of the world, and is undoubtedly the defining public-health crisis of our time.

Viviana et al. [58] reviewed the HIV/AIDS epidemiology, pathogenesis, prevention interventions, and treatment. Research has deepened our understanding of how the virus replicates, manipulates, and hides in an infected person. Although our understanding of pathogenesis and transmission dynamics has become more nuanced and prevention options have expanded, a cure or protective vaccine remains elusive. ART has transformed AIDS from an inevitably fatal condition to a chronic, manageable disease in some settings. This transformation has yet to be realised in those parts of the world that continue to bear a disproportionate burden of new HIV-1 infections and are most affected by increasing morbidity and mortality.

Wang [59] provides an overview of the HIV/AIDS epidemic, scientific research and government responses in China. The unique pattern of the HIV/AIDS epidemic in China–high HIV infection rate among injecting drug users (IDUs) and former plasma donors, but a relatively low overall infection rate in the country provides a window period for taking action. Over the past 10 years, Chinese scientists have conducted several pilot demonstration projects that provided domestic evidence for policy development for controlling AIDS. More recently, the Chinese government has taken bold steps to scale-up HIV testing and counseling, offer free ART to AIDS patients, and expand primary prevention measures such as methadone maintenance and needle exchange programs for drug users and condom promotion for sex workers and men who have sex with men (MSM). The reviewer emphasized that the remarkable achievements in such a short period of time indicate that China is strongly

committed to limiting the epidemic and maintaining a low HIV prevalence into the future.

Karim et al. [60] reviewed the global epidemiology of HIV-AIDS. Although intravenous drug use is the major route of transmission in several countries, sexual transmission is the dominant mode of HIV spread globally, with a concomitant epidemic in infants borne to HIV-infected mothers. The HIV epidemic varies substantially from one geographic area to another, and three broad epidemic categories describe the diversity of features observed globally: low epidemic settings, centralized epidemics, and generalized epidemics. This article describes the modes of HIV transmission, geographic distribution of the evolving AIDS pandemic, and case studies of each of the three types of HIV epidemics, and presents global trends in AIDS and mortality.

In a descriptive cohort study, Schacker et al. [61], examined the clinical and epidemiologic presentation of primary HIV infection in 46 homosexual patients, of which 41 (89%) developed an acute retroviral syndrome. These workers emphasized that primary HIV infection causes a recognizable clinical syndrome that is often underdiagnosed, even in persons enrolled in a program of routine surveillance for HIV infection. In an investigation on the impact of primary HIV infection on the subsequent course of the infection, Pedersen et al. [62] made a prospective study on 86 men in whom seroconversion occurred within 12 months. The group reported an acute illness like glandular fever occurred in 46 (53%) subjects. Three year progression rates to a $CD4^+$ lymphocyte count $<0.5 \times 10^9/l$ and to recurrence of HIV antigenaemia were significantly higher for those who had longlasting primary illnesses than those who had no symptoms or mild illness (75% v 42% and 55% v 14%, respectively). They conclude that the course of primary infection may determine the subsequent course of the infection.

In their study on the rate of development of immunodeficiency in HIV infection in relation to the risk of death, Phillips et al. [63] examined 111 patients with hemophilia who seroconverted to HIV-1 between 1979 and 1985. Patients have been closely followed up clinically and immunologically. Development of immunodeficiency, defined by a $CD4^+$ lymphocyte count falling beneath 0.20 and

0.05 $\times 10^9$/L, and death. Kaplan-Meier estimates suggest that almost half (46%; 95% confidence interval [CI], 26% to 66%) of patients alive 12 years after seroconversion will have a $CD4^+$ lymphocyte count that has remained above 0.05 $\times 10^9$/L. Mortality risk was closely associated with severe immunodeficiency. Only 15% HIV-related death (95% CI, 6% to 25%) occurring before a $CD4^+$ count reached below 0.05 $\times$ 10^9/L. The risk of death was found to be low before the $CD4^+$ lymphocyte count has fallen to 0.05 $\times$ 10^9/L, a count many patients remain above up to 12 years after seroconversion.

Pereyra et al. [64] examined the genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. HIV-specific T cell and antibody responses as well as host genetics were examined in untreated HIV-infected patients who maintain comparatively low plasma HIV RNA levels. HIV-specific T cell and antibody responses as well as host genetics for patients with levels of >10,000 copies/mL were also assessed. The group reported that the individual responses were quite heterogeneous, and none of the parameters evaluated was uniquely associated with the ability to control viremia. Even low-level viremia among HIV controllers was associated with measurable T cell dysfunction, which has implications for current prophylactic vaccine strategies.

Schacker et al. [65] examined the biological and virologic characteristics of acute and very early HIV infection in a prospective, longitudinal cohort study at University of Washington research clinic. Detailed clinical assessments (Plasma HIV-1 RNA levels; quantitative cell cultures; $CD4^+$ cell counts) were done at study entry, biweekly for 1 month, monthly for 2 months, and quarterly thereafter. In the first 30 days after acquisition of HIV, HIV-1 RNA levels varied greatly among participants (range, 27,200 to 1.6 x 10^6 copies per mL of plasma). Levels of HIV-1 RNA decreased by a mean of 6.5% per week for the first 120 days and then increased by a mean of 0.15% per week. $CD4^+$ cell counts decreased by a mean of 5.2 cells/ mm^3 per week for the first 160 days and by a mean of 1.9 cells/ mm^3 per week thereafter ($p < 0.01$). Disease progressed faster in participants who sought medical care for their acute seroconversion syndrome ($p = 0.01$) and those who had high plasma HIV-1 RNA levels 120 to 365 days after acquisition ($p < 0.01$). Peak levels in the first 120

days were not predictive of disease progression. The group reported that the variability in viral RNA levels associated with acute HIV-1 infection is greater than previously appreciated. Within 120 days of acquisition, plasma HIV RNA levels rapidly decrease to an inflection point, after which they gradually increase. Virus-host interactions soon after acquisition seem to have a major influence on the long term outcome of HIV-1 disease.

2.4 HIV Types, Sub-Types and Related Genes

The epidemiology, molecular epidemiology and pathogenesis of HIV infection in India was reviewed by Lakhashe et al. [66]. The year 1986 saw first case of HIV infection as well as first report of AIDS case in India. Since then the epidemic has spread throughout the country. In the recent years there is evidence of epidemic being stabilized with decrease in new infections reported from some parts of the country. HIV viruses circulating in India predominantly belong to HIV-1 subtype C. However, there have been occasional reports of HIV-1 subtype A and B. Matter of concern is reports of A/C and B/C mosaic viruses that are being reported from different parts of the country. The data on HIV drug resistance from India is rather limited. Most of the studies have shown that the virus strains from drug naive patients do not show significant level of drug resistance mutations.

The few immunological studies in Indian patients show that the Indian HIV infected patients show both HIV-specific cytotoxic T lymphocyte (CTL) responses as well as neutralizing antibody response. Mapping of CTL epitopes showed that while Indian patients identify same regions of *gag* antigen as recognized by South African subtype C infected patients, some regions are uniquely recognized by Indian patients. There are very few studies on host genetic factors in India in context with HIV infection. However there are evidences reported of association of host genetic factors such as human leukocyte antigen (HLA) types, haplotypes and HIV disease. Gupta et al. [67] detected HIV-1 A/AE circulating recombinant form (CRF) in India. Rapidly evolving viruses such as HIV-1 develop marked sequence differences in their genome over the course of an epidemic and in individuals infected for longer duration. They emphasized the importance of monitoring various CRFs that are being generated and horizontally spread in the community. This has significant implications for

development of candidate vaccine for India. Sahni et al. [68] observed that HIV-1 subtypes have important implications for the development of candidate vaccine and understanding the possible differences in the transmission and natural history of different subtypes.

The functional and pathogenic implications of genetic variation of global HIV-1 molecular epidemiology with special reference to genetic analysis of HIV-1 subtypes circulating in North India were studied by Neogi et al. [69]. HIV-1 displays extensive genetic diversity globally which poses challenge in designing a suitable antigen/immunogen to provoke desired protective immune response in host. HIV-1 mediated pathogenesis is complex and involves host genes, virus genes and other factors. A number of genetic subtypes have been identified based on sequence variations, largely in envelope region. Different genetic subtypes display variation in amino acid sequences with increasing incidence of subtype B, C, D and mosaic recombinants in India. They can potentially alter the functions of several proteins like Rev, Tat, Vpr, Vif etc and thereby, influence HIV-1 mediated pathogenesis. Recent study has shown that long terminal repeat (LTR) promoter region exhibits novel mosaic structures with segments from B/C Myanmar and India. This indicates rapid evolving nature of HIV-1 and causing epidemics due to existence of multiple subtypes in Indian region. These multiple subtypes show significant differences in various functions (gene activation, cell cycle arrest, RNA binding activities) compared to prototype subtype B genes. These differences may help in better understanding of unique features of HIV-1 epidemic in India.

Kulkarni et al. [70] analyzed the highly complex neutralization determinants on a monophyletic lineage of newly transmitted subtype C HIV-1 *env* clones from India. They found that newly transmitted Indian *env*'s are antigenically complex in spite of close genetic similarity. Delineation of neutralization-associated amino acid signatures provides a deeper understanding of the antigenic structure of HIV-1 *env*.

Banerjea et al. [71] reviewed the host genes that affect progression of AIDS/HIV in India and novel gene therapeutic approaches against HIV. According to their study, the polymorphisms can be utilized as genetic markers for evaluating disease progression and developing effective therapeutics. Their review also describes

the development of anti-viral therapy, involving the use of catalytic nucleic acids like DNA-enzymes and ribozymes and the expression of ribozymes and small interfering -RNA using lentiviral vectors for stem cell based anti-HIV therapy.

Polymorphisms of IL-6 174 G/C, IL-10 -592 C/A and risk of HIV/AIDS among North Indian population was evaluated by Sobti et al. [72]. They applied PCR and restriction fragment length polymorphism (RFLP) techniques to genotype IL-6 and IL-10 and recruited 300 seropositive and an equal number of age- and sex-matched seronegative control subjects for their study. There was statistically no significant variation in the frequencies of IL-6 and IL-10 genotypes among cases and controls. They conclude that the combined effects of the CC of IL-6 and CC of IL-10 might reduce the host's ability to hinder viral replication after infection.

Gupta et al. [4] reviewed the genomic diversity of HIV-1 in India. Phylogenetic analyses, based on the nucleotide or deduced aminoacid sequences of *env*, *gag* and *pol* genes of numerous strains of HIV-1, have revealed three distinct clades of viruses, which have been termed as groups M (main), N (new or non-M, non-O) and O (outlier). Considering the magnanimity of the pandemic, Indian data on epidemiology and distribution of HIV subtypes is relatively frugal. Reasons why genetic subtypes and inter-subtype recombinant genomes need to be analysed are compelling. Molecular epidemiology of HIV-1 strains circulating in different regions of India was carried out using two techniques: heteroduplex mobility assay (HMA) (n=125) and sequencing of full length *gag* genes (n=25)/C2-V3-C3 region of gp120 *env* gene (n=50). All these studies concluded that HIV-1 subtype C was predominant in India accounting for 85-90 % of all isolates. Other subtypes observed included subtypes A, Thai B, E and putative recombinants. Also, it is evident that as the epidemic evolves, subtype C strains are likely to replace subtype Thai B in the Northeast. Lole et al. [73] carried out genetic study on full-length HIV-1 genome from subtype C-infected seroconverters in India, and suggested that recombinants possess one or more gene sequences of HIV-1 derived from different genetic subtypes. The genetic diversity of HIV type 1 subtype C *env* gene sequences from India was studied by Khan et al. [74]. They sequenced and analyzed 28 *env* gene sequences from patients. The samples were collected over a period of 10 years from 1995 to 2004.

According to them, analysis of the phylogenetic relationships among subtype C *env* sequences from six different countries and the current study isolates revealed an overall star-like phylogeny with almost all sequences from India forming a monophyletic lineage. A lower diversity within the immunodominant epitopes was found. They concluded that the data generated from their study should prove valuable for the production of vaccine against subtype C.

The genomic diversity of HIV-1 in India was analysed by Sahni et al. [75]. They determined the HIV-1 subtypes for homologies in the C2-V3-V5 region by HMA in HIV-1 seropositive patients referred to the National HIV/AIDS Reference Centre, All India Institute of Medical Sciences in New Delhi, India. Of the 125 samples analysed, 98 (78.4%) were HIV-1 subtype C, 11 (8.8%) were subtype B', 3 (2.4%) were subtype A and 2 (1.6%) were subtype E. In 11 samples, subtype determination was not clear-cut. Their group concluded that it is possible that these individuals may be infected with recombinant strains of HIV-1 and these findings may have significant implications for the designing and testing of effective HIV-1 vaccine candidate in India.

Gurjar et al. [76] performed molecular epidemiological study on HIV-2 partial envelope gene and LTR sequences from isolates in India. Although, a single full-length sequence of an isolate from India is available in the GenBank, a comprehensive molecular analysis of a large number of HIV-2 isolates would be essential for understanding the genomic heterogeneity of HIV-2 isolates prevalent in the country. In this study, they determined the complete nucleotide and deduced amino acid sequence of a HIV-2 isolate that was obtained by co-culture of infected cells. An extensive full-length genome analysis was performed with sequences available from the GenBank. Analysis of the gene sequences indicated presence of two mutations associated with drug resistance to NRTIs. Mutations associated with protease inhibitors were not observed in the isolate.

Chatterjee [77] correlates the host genetic factors in susceptibility to HIV-1 infection and progression to AIDS. HIV-1 infection has rapidly spread worldwide and has become the leading cause of mortality in infectious diseases. The duration for development of AIDS (AIDS progression) is highly variable among HIV–1 infected

individuals, ranging from 2–3 years to no signs of AIDS development in the entire lifetime. Several factors regulate the rate at which HIV-1 infection progresses to AIDS. Host genetic factors play an important role in the outcome of such complex or multifactor diseases as AIDS and are also known to regulate the rate of disease progression.

Papathanasopoulos et al. [78] reviewed the evolution and diversity of HIV-1 in Africa. The HIV/AIDS pandemic represents a major development crisis for the African continent, which is the worst affected region in the world. Extensive phylogenetic analyses of partial and full-length genome sequences have helped to gain insights into the evolutionary biology and population dynamics of HIV. One of the major characteristics of HIV is its rapid evolution, which has resulted in substantial genetic diversity amongst different isolates, the majority of which are represented in Africa. Genetic variability of HIV and any consequent phenotypic variation poses a significant challenge to disease control and surveillance in different geographic regions of Africa. Their review focuses on the origins and evolution of HIV, current classification and diversity of HIV isolates in Africa and provides an extensive account of the geographic distribution of HIV types, groups, and subtypes in each of the 49 African countries. Numerous epidemiological studies have provided a picture of HIV distribution patterns in most countries in Africa, and these show increasing evidence of the importance of HIV-1 recombinants. They concluded that the current understanding of HIV distribution in Africa is incomplete and inadequately represents the diversity of the virus, and underscores the need for ongoing surveillance.

Ari et al. [79] reviewed whether HIV-1 evolving to a less virulent form in humans. During the rapid spread of HIV-1 in humans, the main (M) group of HIV-1 has evolved into ten distinct subtypes, undergone countless recombination events and diversified extensively. The impact of this extreme genetic diversity on the phenotype of HIV-1 has only recently become a research focus, but early findings indicate that the dominance of HIV-1 subtype C in the current epidemic might be related to the lower virulence of this subtype compared with other subtypes.

Sharp and Hahn [80] analysed the prehistory of HIV-1. Viral archaeology sheds light on the geography and time scale of the early diversification of HIV-1 in humans. HIV-1 must have been spreading through the human population long before AIDS was first described in 1981, but very few strains from this 'prehistoric' period (pre-1980s) have been characterized. Viral sequences from earlier times can provide insight into the early spread of HIV-1, because the rapid rate of evolution of this virus — up to a million times faster than that of animal DNA — means that substantial amounts of sequence change occur in a matter of decades.

Korber et al. [81] analysed the evolutionary and immunological implications of contemporary HIV-1 variation. Evolutionary modeling studies indicate that less than a century has passed since the most recent common ancestor of the HIV-1 pandemic strains; and in that time frame, an extraordinarily diverse viral population has developed. HIV-1 employs a multitude of schemes to generate variants: accumulation of base substitutions, insertions and deletions, addition and loss of glycosylation sites in the envelope protein, and recombination. A comparison between HIV and influenza virus illustrates the extraordinary scale of HIV variation, and underscores the importance of exploring innovative HIV vaccine strategies. Deeper understanding of the implications of variation for both antibody and T-cell responses may help in the effort to rationally design vaccines that stimulate broad cross-reactivity.

Wilkinson and Engelbrecht [82] performed molecular characterization of non-subtype C and recombinant HIV-1 viruses from Cape Town, South Africa**.** HIV was first diagnosed within South Africa in 1982. Homosexual transmission of HIV-1 dominated the epidemic within the country in the early stages of the 1980s. Currently heterosexual transmission of HIV-1 is responsible for the majority of HIV cases in South Africa with subtype C HIV-1 being responsible for an estimated 95% of infections. This study characterized sub-genomic and near full-length sequences of non-subtype C HIV-1 viruses from the Cape Town area. Analysis of sequenced data with the use of sub typing, recombination identification, and tree drawing tools revealed one subtype B and one A1 isolate. The other two isolates were identified as AC and AD recombinants.

Grez et al. [83] by genetic analysis of HIV-1 and HIV-2 mixed infections in India reveals a recent spread of HIV-1 and HIV-2 from a single ancestor for each of these viruses. Phylogenetic tree analysis placed the Indian strains within the C subtype of HIV-1, being most similar to sequences previously found in East and South Africa. The HIV-2 sequences were also closely related to each other, with an overall sequence divergence of between 5.6 and 10.5%. The low level of nucleotide divergence among Indian HIV-1 and HIV-2 sequences suggests a fairly recent introduction of each virus into this population from a single point of entry in each case. The HIV-2 sequences reported here represent the first analysis of Asian HIV-2 strains and confirm the serological pattern previously detected in India. These data show that a substantial spread of HIV-2, together with HIV-1, has appeared outside Africa in a population hitherto unexposed to HIV. These findings imply that further spread of HIV-2 worldwide is to be expected and have important implications for future vaccine and therapy development.

The sequencing primers for HIV-1 and its highly variable genome have been extensively reviewed by Buell et al. [84]. Over several years, multiple, distinct genotypes of HIV-1 were recognized. This finding sparked a multi-national effort to document the full genetic diversity of the virus and to unlock the biological and immunological significance of its genotypic complexity. Significant new discoveries, each incrementally expanding the range of genotypic variation ascribed to HIV-1, have occurred even in the last two years. In addition to at least eight distinct genotypes in the M, or "main" HIV-1 group, a number of rare "O" or outlier forms, and intergenotypic recombinants, combining genetic information from two or more of the major genotypes, have come to light. An early trend towards limited sampling of the genetic information contained in the 10 kilo base pairs (Kb) genome of HIV-1, fostered in part by the widespread use of the PCR to recover HIV-1 genome from clinical samples and virus cultures, resulted in the accumulation of a wealth of short sequences from selected genomic regions and from those genotypes that were first recognized and most accessible for study. Complete genomic sequence information remains scarce or unavailable for many of the genotypes that comprise the prevalent "M" group viruses and, also, for most of the group O and recombinant viruses.

Counter-balancing efforts have begun. Increasingly, investigators are focusing on more complete genomic information and on the relatively less characterized genotypes. Using enriched sources or nested PCR, full-length *gag* or envelope genes are accessible by conventional PCR amplification. With the new awareness that recombinant forms occur at a significant frequency, the impetus for more complete genetic analysis is strengthened; the technical capability to extend PCR amplification to encompass the full HIV-1 genome has appeared concomitantly.

Guimaraes et al. [85] studied the polymorphism of the HIV-1 in Brazil involving genetic characterization of the *nef* gene and has implications for vaccine design. Although only few non-subtype B samples have already been analyzed so far, the CTL epitopes encoded in this region were relatively conserved among the subtypes, with some amino acid signatures mainly in the subtype C samples. Considering the increasing of the non-B HIV-1 subtypes worldwide, in special the subtype C, more data should be generated concerning the genetic and antigenic variability of these subtypes, as well as the study of the impact of such polymorphism in HIV/AIDS vaccine design and testing.

The genetic variability of HIV-1 amongst infected Filipinos and their phylogenetic relationships, temporal introductions and transmission dynamics of identified variants were analyzed by Paladin et al. [86]. They performed PCR amplification and direct sequencing of a 204 base-pair fragment of the *env* C2–V3 region from uncultured peripheral blood mononuclear cells obtained from 51 HIV-1-positive Filipinos infected from 1987 to mid-1996. Their findings demonstrate the presence of multiple genetic subtypes of HIV-1 in the Philippines. The apparent geographic range of previously reported genotypes in South and South-east Asia was extended and has obvious implications for *env*-based antiviral interventions.

Robertson et al. [87] reviewed the viruses characterized over the last few years and found that substantial numbers of HIV-1 isolates have mosaic genomes. According to them, the finding of such hybrid viruses has a number of implications both for attempts at viral characterization (phylogenetic analyses, and tracking of the epidemic), and for concerns about the future genetic diversity of HIV-1 and its impact on vaccine development.

The development of oligonucleotide primers and probes against structural and regulatory genes of HIV-1 and their use for amplification of HIV-1 provirus by using PCR was made by Dawood et al. [88]. They reported new oligonucleotide primers and probes which can be used for the amplification and detection of HIV-1 provirus sequences of not only structural but also regulatory genes. According to them, the primers were very sensitive and specific and can be used for the detection of African and North American strains of HIV-1.

Hutchinson [89] examines the current state of knowledge about HIV/AIDS in terms of its origins, pathogenesis, genetic variation, and evolutionary biology. The HIV virus damages the host's immune system, resulting in AIDS, which is characterized by immunodeficiency, OIs, neoplasms, and neurological problems. HIV is a complex retrovirus with a high mutation rate. This mutation rate allows the virus to evade host immune responses, and evidence indicates that selection favors more virulent strains with rapid replication. While a number of controversial theories attempt to explain the origin of HIV/AIDS, phylogenetic evidence suggests a zoonotic transmission of HIV to humans and implicates the chimpanzee as the source of HIV-1 infection and the sooty mangabey as the source of HIV-2 infection in human populations. New therapies provide hope for increased longevity among people living with AIDS, but the biology of HIV presents significant obstacles to finding a cure and/or vaccine. HIV continues to be a threat to the global population because of its fast mutation rate, recombinogenic effect, and its use of human defenses to replicate itself.

Molecular characterization of HIV-1 and hepatitis C virus in paid blood donors and injection drug users in China was performed by Zhang et al. [90]. Genetic characterization of viral sequences indicated that there are two major epidemics of HIV-1 and multiple HCV epidemics in China. The paid blood donors and transfusion recipients in Henan harbored HIV-1 subtype B, which is similar to the virus found in Thailand, and HCV genotypes 1b and 2a, whereas the IDU in Yunnan, Guangxi, and Xinjiang carried HIV-1 CRFs 07 and 08, which resemble those in India, and HCV genotypes 1b, 3a, and 3b. According to them, the epidemics of HIV-1 and HCV infection in China are the consequences of multiple introductions. The distinct

distribution patterns of both the HIV-1 and HCV genotypes in the different high-risk groups are tightly linked to the mode of transmission rather than geographic proximity. The group stressed that their findings provide information relevant to antiviral therapy and vaccine development in China and should assist public health workers in implementing measures to reduce the further dissemination of these viruses in the world's most populous nation.

2.5 Biochemical Markers

Karsdal and Kasper [91] assessed the importance of biochemical markers as tools to increase efficiency in drug development. They suggested that an optimal combination of biochemical markers together with clinical risk factors and imaging will most likely provide a useful toolkit to identify patients at high risk, ensuring inclusion of the appropriate patients for clinical studies, identification of patients who will benefit the most from treatment and early intervention of a pathological condition before disease manifestation.

2.5.1 Liver Function Tests (LFTs)

Mata-Marin et al. [92] developed a correlation between HIV viral load and aminotransferases as liver damage markers in HIV infected naive patients. Patients with antiviral treatment experience, hepatotoxic drugs use or co-infection were excluded. The group reported that there is an association between HIV viral load and aminotransferases as markers of hepatic damage; and emphasized on improved recognition, diagnosis and potential therapy of hepatic damage in HIV infected patients.

Ejilemele et al. [93] demonstrated the pattern of abnormal liver enzymes in HIV patients presenting at the University of Port Harcourt Teaching Hospital, Port Harcourt. Abnormalities of LFT have been shown to be common in HIV/AIDS in developed countries. In their study, they recruited a total of one hundred and twenty nine (129) cases. One hundred and thirteen patients (87.6%) had abnormalities of their LFTs. The group concluded that abnormalities of liver enzymes are common in patients with HIV in that environment and emphasized on characterization to assess the nature of abnormality and appropriate management.

Feldmana, et al. [94] evaluated whether the serum albumin level is associated with survival in HIV-1 infected women. The group concluded that baseline serum albumin level is an independent predictor of mortality in HIV-1-infected women. The serum albumin level may be a useful additional marker of HIV-1 disease progression, particularly among asymptomatic women with little or no evidence of immune-suppression.

In a cross sectional study, Ebuehi et al. [95] evaluated the blood chemistry and platelet serotonin uptake as alternative method of tracking HIV/AIDS. The serum glutamic-oxaloacetic transaminase (SGOT) of non- ARV subjects was the only blood chemistry parameter that showed any significant variation from normal ($p<0.05$). The data obtained showed that SGOT activity is significantly increased in HIV/AIDS patients in a manner that is disease stage related. However, serum glutamic-pyruvic transaminase (SGPT), bilirubin, triglycerides, amylase, serum creatinine, and alkaline phosphatase showed no significant variation from normal values. Platelet serotonin uptake of HIV subjects was not significantly different from the control.

In two separate studies, Emejulu et al. [96] in Nigeria and Lucien et al. [97] in Cameroon examined the hepatotoxicity of ARV drugs in HIV seropositive patients. The Nigerian group claims that their study affirms the potential risk of hepatotoxicity for HIV seropositive patients on ARV drugs and calls for continuous monitoring of ARV administration so as to prevent fatal effects of hepatotoxicity while the Cameroon group concluded that HAART is associated with low level hepatotoxicity at therapy initiation, regardless of drug class or combination. Ofotokun et al. [98] examined the liver enzymes elevation (LEE) and immune reconstitution among treatment-naive HIV-infected patients instituting ART. LEE complicates ARV therapy, and because the strongest risk factor for ARV-related LEE is HBV/HCV co-infection, it is speculated that ARV-related LEE may be a form of immune reconstitution disease. Their finding suggests that ARV-related liver enzyme elevation may be related in part to immune reconstitution, as measured by changes in $CD4^+$ T-cell counts.

Price and Thio [99] reviewed the liver disease in the HIV–infected individual. Their review discussed the causes of liver disease in the HIV-infected population in

the ART era, including chronic HCV, chronic HBV, medication related hepatotoxicity, alcohol abuse, non-alcoholic fatty liver disease, and AIDS related liver diseases.

Fokunang et al. [100] performed a pilot longitudinal study of hepatotoxicity and nephrotoxicity in HIV patients on HAART at a day care clinic at the Yaounde Central Hospital, Cameroon, between April and July 2008. For hepatotoxicity evaluation, with aspartate aminotransferase (AST) study, 5.8% of patients showed degree 1 and 1.3% of patients had degree 2 changes. In alanine aminotransferase (ALT), 3.70% of patients showed degree 1 and 3.95% degree 2 changes. The ARV regimen that was involved in most of these changes was Zidulam N. The ARV regimen administered to patients during the study did not really affect creatinine levels and hence showed no risk of toxicity to the kidneys.

Palanisamy et al. [101] analysed the changes in $CD4^+$ cell count, lipid profile and liver enzymes in HIV infection and AIDS patients. The study population consisted of 150 subjects, age and sex-matched and divided into three groups [control subjects (n=50), HIV infected (n=50) and AIDS patients (n=50)]. The group observed a significant reduction in $CD4^+$ cell count in HIV/AIDS patients when compared to control subjects. Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were found to be decreased significantly in HIV/AIDS patients when compared with normal counterparts. On the other hand, the levels of triglyceride (TG) and very low-density lipoprotein cholesterol (VLDL-C) were markedly elevated in HIV/AIDS patients compared to normal subjects. The activities of serum AST, ALT and alkaline phosphatase (ALP) observed in HIV/AIDS patients were significantly higher than in the control group. Further, the above mentioned haematological and biochemical variables were found to be affected more significantly in AIDS patients when compared with HIV infected subjects. The group concluded that $CD4^+$ cell count, lipid profile and liver enzymes can be a good index of disease progression in HIV infection and AIDS patients. Introduction of effective combined ART has made HIV infection a chronic illness. Substantial reductions in the number of AIDS-related deaths have been accompanied by an increase in liver-related morbidity and mortality

due to co-infection with chronic HBV and HCV. Joshi et al. [102] reviewed the increasing burden of liver disease in patients with HIV infection. Introduction of effective combined ART has made HIV infection a chronic illness. Substantial reductions in the number of AIDS-related deaths have been accompanied by an increase in liver-related morbidity and mortality due to co-infection with chronic HBV and HCV. Increases in non-alcoholic fatty liver disease and drug-induced hepatotoxicity, together with development of hepatocellular carcinoma, also potentiate the burden of liver disease in individuals with HIV infection. The group provides an overview of the key causes, disease mechanisms of pathogenesis, and recommendations for treatment options including the evolving role of liver transplantation.

Puoti et al. [103] assessed incidence, risk factors, histology, and outcome of severe hepatotoxicity during combination ART. Their study provides estimates of severe hepatotoxicity and liver failure in a large population based setting where HCV co-infection is highly prevalent and provides indications that liver damage may be caused by immune reconstitution and related exacerbation of viral hepatitis. They advocated for a mandatory follow-up for hepatotoxicity, when ART is initiated in patients with <200 $CD4^+$ T cells/mm3 and suggested that anti-hepatitis pre- or co-medication could be an effective preventive or curative measure. Yimer et al. [104] assessed and compare the prevalence, severity and prognosis of anti-TB drug induced hepatotoxicity in HIV positive and HIV negative TB patients in Ethiopia. The group reported that anti-TB drug induced hepatotoxicity is a major problem in HIV-associated TB with a decline in immune status and that there is a need for a regular biochemical and clinical follow up for those patients who are at risk.

Hepatotoxicity has been demonstrated to be associated with ART. Becker [105] reviewed that hepatotoxicity in HIV-1 infected patients was significantly associated with co-infection with viral hepatitis. The elevated baseline ALT levels predicted subsequent hepatotoxicity. According to him, there was a low incidence of long-term hepatotoxicity in different studies and no consistent association between a particular drug or drug class.

Oguntibeju and Banjoko [106] assessed the activities of liver enzymes in HIV/AIDS patients in Lagos, Nigeria. They reported that the activities of serum alanine-aminotransferase ($p<0.01$), aspartate-aminotransferase ($p<0.03$) and alkaline phosphatase ($p<0.001$) observed in HIV infected/AIDS patients were significantly higher than those in the reference group. Non significant differences were observed with regards to sex in the serum levels of the three afore-mentioned liver enzymes. They conclude that increase in the three liver enzymes is most likely due to impairment/involvement of the liver in HIV infection and the enzymes may be useful markers for HIV and AIDS.

To study and understand the function of liver and simplify the interpretation of liver function tests, Thapa and Walia [107] described their interpretation with algorithms. The Model for End-Stage Liver Disease (MELD) score is widely used to prioritize patients for liver transplantation. Portea et al. [108] underscores the problems and solutions involved in the International Normalized Ratio (INR) in the MELD Score. One of the pitfalls of the MELD score is the inter-laboratory variability in all three components of the score (INR, bilirubin, creatinine). The inter-laboratory variability in the INR has the largest impact on the MELD score, with a mean difference of around 5 MELD points in most studies.

2.5.2 Kidney Function Tests (KFTs)

Emejulu et al. [109] designed a study to assess the possible incidence of electrolyte abnormalities and renal impairment in treatment-naive HIV patients in an urban community in Nigeria. They reported that serum urea concentrations were significantly increased ($p<0.05$) in both male and female patients. They indicated that renal function may be impaired in HIV positive patients who have not been placed on any ARV drug. Esezobor et al. [110] found correlation between kidney function and HIV-infection in children of Lagos, Nigeria, using Filler's serum cystatin C-based formula. They reported that HIV-infected children in Nigeria have higher serum cystatin C level and lower eGFR compared to age and sex matched controls.

Emem et al. [111] performed an assessment of prevalence, clinical features and risk factors in renal disease in HIV-seropositive patients in Nigeria. They

observed a high prevalence of renal disease (proteinuria and/or elevated serum creatinine), which was present in 152 (38%) of the patients. In subjects with and without nephropathy, they reported significant differences in age, body mass index (BMI), serum cholesterol, serum albumin and $CD4^+$ counts, suggesting that these parameters may be risk factors for nephropathy. Further, the histological samples revealed mainly focal glomerulosclerosis with glomerular collapse. Brook and Miller [112] reviewed the current knowledge on the etiology, pathology, diagnosis, and treatment of HIV associated nephropathy (HIVAN). As per their review, they suggest that HIVAN will respond to HAART with a dramatic improvement in renal function and thus described HIVAN as a treatable condition. This condition should be actively sought in HIV infected patients if they are to receive the benefits of therapy. Kamga et al. [113] indicate that renal function is not affected by the seropositivity status of individuals. Similarly, Afhami et al. [114] in their study about renal disorders in HIV-infected patients in Iran, reported no electrolyte imbalance, proteinuria, or renal failure in HIV-infected patients. They suggest that renal disorder is infrequent in Iranian HIV-infected patients.

Winston et al. [115] proposed that HIVAN is a late and not early manifestation of HIV-1 infection. HIVAN can be the initial presentation of HIV-1 infection. HIVAN develops late, not early, in the course of HIV-1 infection following the development of AIDS. This likely account for the poor prognosis noted in previous publications and has implications for pathogenesis. In addition, given the detectable viral RNA levels, HAART is indicated in HIVAN. HAART may improve survival as well as alter the natural history of HIVAN. Szczech et al. [116, 117] studied the association between renal disease and outcomes among HIV-infected women receiving or not receiving ART and examines the risk factors for proteinuria and renal failure. The group concluded that proteinuria and an elevated creatinine level were associated with an increased risk of death and development of AIDS defining illness. Their analysis also establishes the associations between both increasing HIV RNA level and decreasing $CD4^+$ lymphocyte count with the presence of proteinuria and occurrence of renal failure. These associations may reflect the direct role of the kidney in modulating

HIV disease, or they may act as markers of greater co-morbidity. Additionally, it demonstrates an association between proteinuria and a positive hepatitis C antibody.

Cavalcante et al. [118] studied the prevalence of persistent proteinuria in stable HIV/AIDS patients and its association with HIV nephropathy. They examined the prevalence of and associated risk factors for precipitation, along with the prevalence of HIVAN among AIDS patients. They reported that prevalence of persistent proteinuria was 5.6% (95% CI = 3.6 to 8.3%), and it was significantly associated with a low $CD4^+$ lymphocyte count ($p<0.048$).

Daugas et al. [119] reviewed the HAART-related nephropathies in HIV-infected patients. A growing number of observations suggest that the beneficial effects of HAART also include improvement of HIV-related renal complications. However, some ARV drugs may have renal and life-threatening side-effects, especially if underlying renal abnormalities exist. The role of ART in reducing the incidence of OI and death after HIV infection was reviewed by Posta and Holt [120]. Their review focuses on recent progress in our understanding of the clinical epidemiology of HIV associated kidney disease.

To correlate $CD4^+$ counts with albuminuria and glomerular lesions in patients infected with HIV, Janakiraman [121] screened 100 patients over nine months for albuminuria by urine dip stick method, and performed renal biopsy on patients with albuminuria 2+ or more. Albuminuria was observed in 29 (27%) patients, and it revealed a significant negative correlation with $CD4^+$ count ($p < 0.01$). Patients with $CD4^+$ cells < 350 cells/mm disclosed a 3.5 fold increased risk of albuminuria as compared with patients with $CD4^+$ > 350 cells/mm. There was no significant correlation between proteinuria and the duration of infection from the time of diagnosis. Albuminuria also demonstrated a significant negative correlation with the levels of hemoglobin ($p<0.05$). In addition, low numbers of $CD4^+$ cells were associated with lower levels of hemoglobin ($p<0.001$). Only 10 patients received renal biopsies, and the results revealed HIVAN in 7 (70%) patients, chronic tubulointerstitial nephritis in 1, membranous glomerulopathy in 1, and diffuse proliferative glomerulonephritis in 1. Acute renal failure was present in 5 patients, of

whom four had a pre renal component and one had multiorgan dysfunction syndrome. The report concluded that both proteinuria and HIVAN are common in HIV infected patients with proteinuria having a negative correlation with the $CD4^+$ counts and hemoglobin levels.

Duggan [122] advocated that the activities of enzymes in plasma should be measured at 37°C. There are considerable differences of opinion as to the most desirable temperature at which activities of enzymes in plasma should be measured. Debate centers around 25°C because of its widespread use in physico-chemical measurements, 30°C because of its supposed technical convenience, and 37°C because of its physiological significance. There is, however, widespread agreement that the activities of intracellular enzymes, and of enzymes having a natural function in plasma, be measured at 37°C. Perhaps because estimations of the activities of those enzymes in plasma that have leaked from damaged tissues have been subject to almost continuous modifications, temperature has simply become just another variable to take into account. In this article the researcher describes the relevance of a temperature near 37°C to the activities of all enzymes in man, intracellular and extracellular. The report concludes that 37°C is to be favored as the temperature for assaying all parameters in human biochemistry affected by temperature. These include not only enzyme activities but also pH, blood-gas equilibria, binding data and infact, all kinetic activities in the body.

2.5.3 Lipid Function and Blood Glucose Test

Oh and Hegele [123] reviewed the pathogenesis and treatment of HIV-associated dyslipidaemia. Key Pathogenic mechanisms for variation in dyslipidaemia include effects of the virus itself, effects of the antiretroviral drugs on key metabolic pathways, and drug-associated adipose repartitioning with subsequent development of insulin resistance and associated metabolic derangements. Kumar and Sathian [124] assessed lipid profile in patients with HIV/AIDS without ART. Significantly higher levels of triglycerides, LDL-cholesterol, LDL / HDL-C, TG/HDL-C and CD4/CD8 ratio were observed along with decline in $CD4^+$ cells/µL, CD-8 cells/ µL (P=0.0001). Further there was a strong correlation between $CD4^+$ cells/ µL and TG, LDL-C. Triglycerides and LDL-C level also increased proportional to the increase in $CD4^+$

cells/ µL. The group concluded that the changes in lipid profile can be a good index of disease progression in HIV infection.

Iffen et al. [125] examined the lipid profile of HIV-positive patients in Calabar, Nigeria and reported that lipid profile monitoring is as necessary as $CD4^+$ T lymphocyte count monitoring for the well being of HIV patients in this locality. The potential risk of serum lipid profiling in HAART-naive HIV positive patients in Ghana was examined by Obirikorang et al. [126]. Disorders of lipid metabolism have been described in patients with HIV infection before the introduction of HAART, including increased serum TG levels and decreased cholesterol levels observed at various stages of HIV infection. Serum lipid profile in HIV infected patients were studied by Khiangte et al. [127] at the Regional Institute of Medical Sciences, Imphal, to find out correlation between the changes in lipid profile and the progress of HIV infection. Their results showed that lipid profile was altered in HIV infected/AIDS patients and that monitoring of lipid profile in HIV/AIDS patients may be useful in assessing the disease progression and management in the absence of $CD4^+$ count facility.

El-Sadr et al. [128] evaluated the effects of HIV disease on lipid, glucose and insulin levels in a large ARV-naive cohort. Both HIV disease and demographic characteristics were found to influence lipid values and glucose homeostasis in the absence of ARV treatment. More advanced HIV disease was associated with less favorable lipid and glucose homeostatic profiles. The independent association between HIV RNA levels and various lipid parameters suggests that viral replication had a direct effect on lipid levels. Interpretation of the effects of various HIV treatment regimen and drugs on metabolic parameters must take into account the stage of HIV disease and the demographic characteristics of the population studied.

Sekhar et al. [129] investigate the abnormalities in dietary fat disposal in the pathogenesis of hypertriacylglycerolemia in HIV lipodystrophy syndrome (HLS). They observed significantly elevated concentrations of fasting plasma triacylglycerols in HLS patients and concluded that patients with HLS have key defects that markedly impair postprandial disposal and storage of chylomicron-triacylglycerols. These defects contribute significantly to hypertriacylglycerolemia in HLS.

The lipid profile in HIV/AIDS patients in Nigeria was studied by Adewole et al. [130]. The group reported that abnormality of serum lipid is common among treatment naive HIV patients seen in Nigeria, while NNRTI regimen is associated with elevation of HDL and some stabilization of TC and TG. Reeds et al. [131] studied the alterations in liver, muscle, and adipose tissue insulin sensitivity in men with HIV infection and dyslipidemia. They evaluated insulin action in skeletal muscle, liver, and adipose tissue in HIV-infected men with dyslipidemia. The group concluded that dyslipidemia in HIV-infected men is indicative of multi-organ insulin resistance, and circulating adipokines may be important in the pathogenesis of impaired insulin action.

Wijk et al. [132] examined the *in-vivo* evidence of impaired peripheral fatty acid trapping in patients with human immunodeficiency virus-associated lipodystrophy. They postulated that patients with HIV-lipodystrophy have impaired adipose tissue free fatty acid (FFA) trapping and, consequently, increased hepatic FFA delivery. Riddler et al. [133] analysed the impact of HIV infection and HAART on serum lipids in men. They concluded that before treatment, HIV infection results in substantial decreases in serum TC, HDL-C, and LDL-C levels. Subsequent HAART initiation is associated with increases in TC and LDL-C but little change in HDL-C. Increases in TC and LDL-C observed after about 3 years of HAART possibly represent a return to pre-infection serum lipid levels after accounting for expected age-related changes.

2.6 Molecular Markers and $CD4^+$ cell count

Mellors et al. [134] compared clinical, serologic, cellular, and virologic markers for their ability to predict progression to the AIDS and death during a ten year period. Plasma viral load strongly predicts the rate of decrease in $CD4^+$ lymphocyte count and progression to AIDS and death, but the prognosis of HIV infected persons is more accurately defined by combined measurement of plasma HIV-1 RNA and $CD4^+$ lymphocytes. In a similar study Brumme et al. [135] studied the impact of select immunologic and virologic biomarkers on $CD4^+$ cell count decrease in patients with chronic HIV-1 subtype C infection from Sinikithemba Cohort, Durban, South Africa. They assessed the relationship of these responses,

along with established HIV-1 biomarkers, with rates of $CD4^+$ cell count decrease in individuals infected with HIV-1 subtype C and observed that, the rate of $CD4^+$ cell count decrease in a longitudinal population-based cohort of 300 therapy naive, chronically infected adults with baseline $CD4^+$ cell counts 1200 cells/mm^3 and plasma viral loads 1500 copies/ mL over a median of 25 months of follow-up.

In a review of surrogate markers in HIV disease, Peto [136] describes their use as an attractive method of assessing the efficacy of new treatments more quickly than by using clinical end-points. Results from 14 randomized controlled trials of nucleoside analogues are used to compare the comparative changes of $CD4^+$ counts with the differential rates of progression to AIDS and differences in survival. There was some correlation between $CD4^+$ count changes and development of AIDS, particularly in the short term trials. In contrast, there was little correlation between $CD4^+$ counts and overall survival. They concluded that no surrogate marker has yet been shown to be useful in predicting the efficacy of anti-HIV treatment. Further they advocated that until surrogate markers are validated against the results from long term clinical trials, they should only be used to screen new drugs warranting further study rather than to draw conclusions on the clinical efficacy of new treatments.

Paintsil et al. [137] examined the utility of absolute $CD4^+$ T Lymphocyte count as a surrogate marker of pediatric HIV disease progression. Traditionally in pediatric HIV, the $CD4^+$ T lymphocyte percent is used in monitoring disease progression due to the variability in absolute $CD4^+$ T lymphocyte numbers. Because of the high cost of equipment, sophisticated and delicate technology, most laboratories in resource-limited settings use simple protocols that enumerate only the absolute $CD4^+$ T lymphocyte counts. The group analyzed the $CD4^+$ T lymphocytes and HIV viral load over a 10-year period (1996 to 2006) of 97 HIV-infected children enrolled in the Yale Prospective Longitudinal Pediatric HIV Cohort using generalized linear mixed models. Both $CD4^+$ T lymphocytes and HIV viral load were assessed at baseline and every 2-3 months. They determined that absolute $CD4^+$ T lymphocytes count was just as reliable at monitoring pediatric HIV as $CD4^+$ T lymphocyte percentage. ART, regardless of the regimen used, was associated with higher $CD4^+$ T lymphocytes count ($p < 0.01$). The presence of other infections was associated with

lower $CD4^+$ T lymphocyte count ($p=0.01$) and higher viral load ($p<0.01$, respectively). They advocated that in situations where determination of $CD4^+$ T lymphocyte percentages is not readily available, the absolute count may provide an affordable and accessible laboratory surrogate marker of HIV disease progression in children.

Laboratory markers that predict HIV-1 disease progression include plasma viral burden, $CD4^+$ T cell count, and CD38 expression on CD8 T cells. To better understand whether the predictive value of these markers is dependent on how long an individual has been infected, Giorgi et al. [138] examined the predictive value of immunologic and virologic markers in a multicenter AIDS cohort study. The group found that HIV RNA and CD38 levels were similarly predictive of AIDS early on compared with a relatively weaker $CD4^+$ cell count signal. Later in the course of infection, CD38 level remained the strongest predictive marker and $CD4^+$ cell count registered a marked increase in prognostic power. Among untreated individuals, there was little difference in prognosis (median time to AIDS) associated with given marker values regardless of infection duration. The group emphasized that the data provide a unique historical look at the predictive value and prognostic significance of HIV-1 disease markers at different stages of infection in a large cohort, with direct relevance to current patients who are untreated or for whom treatment is ineffective. The serum beta-2 microglobulin level is elevated in HIV-infected people and has been shown to be a better predictor of HIV infection status than $CD4^+$ counts and a better predictor of survival.

Piwowar et al. [139] studied the beta-2 microglobulin values among HIV-negative, HIV-positive asymptomatic, and HIV-positive symptomatic Ugandans. Mean serum beta-2 microglobulin levels among healthy HIV-seronegative and asymptomatic and symptomatic HIV-seropositive Ugandans were found to be 2.35, 3.75, and 5.06 mg/liter, respectively ($p < 0.001$). The upper limit of the normal range (3.5 mg/liter) is higher in this African population than that reported elsewhere.

Since the first discovery of severe immunodeficiency among previously healthy homosexual men, the knowledge on the HIV virus and the pathogenic mechanisms induced by the virus are extensive, though still incomplete. Katzenstein

[140] reviewed the molecular biological assessment methods and understanding of the course of the HIV infection and emphasized that the ability to quantify viral load and to perform sequence analyses represent valuable tools both for understanding the pathogenic actions of the virus and for the clinical monitoring of HIV-infected patients. The optimal usage of these tools in the clinical setting, however, still remains to be defined. The researcher highlight that the progresses obtained have unfortunately been restricted to the Western World and the calamities of HIV is spreading and worsening in the developing world. The progress in the development of a vaccine has been disappointing and it is urgently necessary that the progresses obtained within the fields of prevention and treatments are translated into useful strategies in the parts of the world mostly affected by the HIV pandemic. Over the past several years, the development and application of molecular diagnostic techniques has initiated a revolution in the diagnosis and monitoring of infectious diseases.

Tang et al. [141] reviewed the molecular diagnostics of infectious diseases. Microbial phenotypic characteristics, such as protein, bacteriophage, and chromatographic profiles, as well as biotyping and susceptibility testing, are used in most routine laboratories for identification and differentiation. Nucleic acid techniques, such as plasmid profiling, various methods for generating restriction fragment length polymorphisms, and the PCR, are making increasing inroads into clinical laboratories. PCR-based systems to detect the etiologic agents of disease directly from clinical samples, without the need for culture, have been useful in rapid detection of unculturable or fastidious microorganisms. Additionally, sequence analysis of amplified microbial DNA allows for identification and better characterization of the pathogen. Subspecies variation, identified by various techniques, has been shown to be important in the prognosis of certain diseases. Other important advances include the determination of viral load and the direct detection of genes or gene mutations responsible for drug resistance. Increased use of automation and user-friendly software makes these technologies more widely available. Elucidating virus-host interactions responsible for HIV-1 transmission is important for advancing HIV-1 prevention strategies. To this end, single genome amplification

and sequencing of HIV-1 within the context of a model of random virus evolution has made possible for the first time an unambiguous identification of transmitted/founder viruses and a precise estimation of their numbers.

Excoffier et al. [142] presented a framework for the study of molecular variation within a single species. Information on DNA haplotype divergence is incorporated into an analysis of variance format, derived from a matrix of squared distances among all pairs of haplotypes. This analysis of molecular variance (AMOVA) produces estimates of variance components and F-statistic analogs, reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision. The method is flexible enough to accommodate several alternative input matrices, corresponding to different types of molecular data, as well as different types of evolutionary assumptions, without modifying the basic structure of the analysis. The significance of the variance components and F-statistics is tested using a permutational approach, eliminating the normality assumption that is conventional for analysis of variance but inappropriate for molecular data. The group showed that population subdivisions are better resolved when some measure of molecular differences among haplotypes is introduced into the analysis. At the intra-specific level, however, the additional information provided by knowing the exact phylogenetic relations among haplotypes or by a non-linear translation of restriction site change into nucleotide diversity does not significantly modify the inferred population genetic structure. According to them, the AMOVA treatment is easily extended in several different directions and it constitutes a coherent and flexible framework for the statistical analysis of molecular data.

Saxena et al. [143] find out normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians. A national task force was constituted by the Indian Council of Medical Research (ICMR), to define reference ranges for several lymphocyte sub-populations in healthy Indians. The task force comprised six centres in different locations in India and analysed by flow cytometry, CD3, CD4, CD8, CD19, CD16 and CD56 populations in peripheral blood samples from a total of 1027 healthy Indians. National means of percentages of different sub-populations of lymphocytes were determined as follows: 68.65% (CD3); 37.10%

(CD4); 34.04% (CD8); 14.67% (CD19); 14.58% (CD16) and 12.44% (CD56). Mean CD4/CD8 ratio was 1.2 for all samples. Significant geographical differences were found in percentages of CD4 and CD8 sub-populations and consequently in their ratios. In southern states, especially Tamil Nadu and Kerala, CD4/CD8 ratios were significantly lower than in northern and western parts of India. They presented a detailed statistical analysis of the data and effects of variables like age, sex, smoking, consumption of alcohol, nutritional status, and self perception of state of health on lymphocyte subpopulations.

Ray et al. [144] in their study counted CD4/CD8 lymphocyte in healthy, HIV-positive individuals & AIDS patients. The enumeration of CD4 and CD8 positive cells, surrogate markers for HIV disease progression is helpful in management and follows up of immune compromised HIV positive patients. In a cross-sectional study, they determine the reference range of T-cell subsets in healthy North Indians and compared the values with those in HIV-positives. Their findings on T-cell subset reference ranges of normal healthy North Indians validate the utility of determination of $CD4^+$ cell count as a useful predictor of AIDS in Indian conditions and confirm that a significant per cent of AIDS patients had $CD4^+$ cell count below 200/µl.

Uppal et al. [145] studied the normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. Measurement of T-cell subsets is important in India for evaluating disease stage and progression in individuals with the HIV. They conducted this study to provide normal ranges of absolute and percentage values of CD4 and CD8 T-lymphocyte subsets and the ratio of CD4 to CD8 in normal Indian adults. When compared with other published series, the CD4 and CD8 values in healthy Indians were no different from those reported in the West. These observations have important clinical implications for the use of T-lymphocyte subset measurements in India, especially in the management of HIV infection.

Oladepo et al. [146] established the reference values of CD4 and CD8 lymphocyte subsets in healthy Nigerian adults in a 2006 study which enrolled 2,570 apparently healthy HIV-negative adults from the six geopolitical zones in the country. The majority (64%) of the participants had $CD4^+$ counts within the range of 501 to

1000 cells/ml. The reference range for CD4 was 365 to 1,571 cells/ml, while the reference range for CD8 was 145 to 884 cells/ml. Alemnji et al. [147] examined the associations between $CD4^+$ cell counts and clinical presentations among HIV/AIDS patients in Cameroon. Statistically significant associations were established between low $CD4^+$ cell counts and various pathologies. They reported that majority of the subjects get to know their HIV status very late, already developing clinical signs and symptoms of AIDS with very low $CD4^+$ cell counts and emphasized the need for improvement of early HIV diagnosis strategy through education and voluntary counseling among populations in Africa.

McGovern et al. [148] assessed the impact of cirrhosis on $CD4^+$ T cell counts in HIV-seronegative patients. The group hypothesized that, in patients with advanced liver disease, low $CD4^+$ T cell counts may occur secondary to portal hypertension and splenic sequestration, regardless of the presence or absence of HIV infection. They reported that cirrhosis is associated with low $CD4^+$ T cell counts in the absence of HIV infection and suggested that their findings have significant implications for the use and interpretation of absolute $CD4^+$ T cell counts in HIV infected patients with advanced liver disease.

Ajayi et al. [149] evaluated the $CD4^+$ cell counts in adults with HIV infections presenting at the medical department of the Federal Medical Centre, Ido-Ekiti, Nigeria during July-December 2006, which was recently upgraded to serve as the only center for HIV/AIDS referral, diagnosis and treatment in Ekiti State. A total of 87 patients comprising of 54 (62.1%) females and 33 (37.9%) males had their $CD4^+$ T-Lymphocytes cell counts evaluated within their first week of presentation. The total mean $CD4^+$ cell count was 230.7 ± 311.9 cells/ µL. The mean $CD4^+$ cell count of females was 212.17 ± 264.96 cells/µL, while that of males was 261.0 ± 389.19 cells/mL. This difference was not significant ($p = 0.4876$). Majority of the patients [75, (86.2%)], had $CD4^+$ cell count < 350 cells/ µL, comprising of 48 females and 27 males. Of the 75 patients, 57 (76%) had a $CD4^+$ cell count < 200 cells/µL (33 females vs 24 males). At the time of HIV diagnosis, majority of the patients had $CD4^+$ cells count < 200 cells/ µL. This was consistent with a relatively advanced disease. More

women than men in the population were found positive for HIV. The group emphasized on more sustained and vigorous awareness campaigns embarked upon in the HIV propaganda in the Ekiti State on one hand and Nigeria on the other hand to bring down HIV/AIDS.

Taylor et al. [150] analyze the data from the Los Angeles portion of the multicenter AIDS cohort study to find the $CD4^+$ T-cell number at the time of AIDS. According to them, the median $CD4^+$ T cell count at the time of AIDS is 67 cells/mm-^{3}. The authors estimate the correlation between the $CD4^+$ T-cell number at the time of AIDS and the $CD4^+$ T-cell number prior to HIV infection to be 0.71 with a 95% confidence interval of 0.21-0.94. They suggest that the very high correlation is suggestive of biologic hypotheses concerning possible control of the circulating $CD4^+$ T-cell number and the high correlation can also be useful in determining when to start prophylactic treatment.

Yarchoan et al. [151] investigated the relation between $CD4^+$ count and the immediate hazard of dying in patients receiving zidovudine (azidothymidine [AZT])-based ART. 55 patients with HIV infection and either AIDS or severe ARC who were followed for as many as 4 years while they received ART were studied in a retrospective analysis of a cohort of patients in United States. $CD4^+$ counts were measured. Ten patients are known to be alive and 1 was lost to follow-up. Of the 44 patients who are known to have died, the CD4 range was known within 6 months of death in 41. All but 1 of these 41 assessable deaths occurred in patients whose $CD4^+$ counts were known to have fallen below 50 $CD4^+$ cells/mm^3 ($p < 10^{-10}$). The hazard of dying in the cohort ranged from 0 deaths/patient-month (95% CI, 0 to 0.008 deaths/ patient-month) in patients with 200 or more $CD4^+$ cells/ mm^3 to 0.07 deaths/patient-month (CI, 0.050 to 0.094 deaths/patient-month) in patients with fewer than 50 $CD4^+$ cells/mm^3. For the patients who died and whose cases were assessable, the mean of the last three $CD4^+$ counts obtained before death was 7.7 $CD4^+$ cells/mm^3 (CI, 0.9 to 63.3 cells/mm^3). The median survival of patients once their $CD4^+$ counts fell below 50 $CD4^+$ cells/mm^3 was 12.1 months (CI, 7.2 to 19.4 months). Nearly all deaths occurred in patients with fewer than 50 $CD4^+$ cells/mm^3. These findings may have implications

in the monitoring of patients with AIDS and in the use of $CD4^+$ count as a clinical trials end point for the ART of HIV infection.

2.6.1 Random Amplified Polymorphic DNA (RAPD)

Characterisation of genomic DNA through identification and determination of random polymorphic markers has proved a powerful application of DNA technology. Random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR) is a DNA fingerprinting technique used to detect genomic polymorphisms. Williams et al. [152] reviewed the concept of DNA polymorphisms amplified by arbitrary primers for their usefulness as genetic markers. Molecular genetic maps are commonly constructed by analyzing the segregation of restriction fragment length polymorphisms (RFLPs) among the progeny of a sexual cross. The researchers described a new DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence and suggested that these polymorphisms be called RAPD markers, after Random Amplified Polymorphic DNA.

Aikhionbare et al. [28] studied the application of RAPD – PCR for genomic analysis of HIV-1infected individuals. They employed sixteen different RAPD – PCR 10-mer primers to amplify DNA from the peripheral blood mononuclear cells (PBMC) of 80 HIV-1-infected individuals. These individuals were previously identified as either heterozygotes _qrD32 and homozygotes _qrq for the CCR5 locus by PCR with gene specific primers. Four of the sixteen randomly selected RAPD primers produced distinguishable banding profiles between CCR5 _qrD32. heterozygotes and CCR5 _qrq homozygotes. Direct sequencing of some RAPD-PCR products obtained with one of the four RAPD primers that were tested yielded clearly readable, but limited sequences, which were similar to portions of the previously published sequences for _qrq. homozygotes _98% similarity and _qrD32 heterozygotes _87% similarity of the CCR5 alleles. Thus, the RAPD-PCR technique may be useful for the identification of human molecular markers that may correlate with susceptibility to HIV-1 infection, or differences in disease progression among HIV-1 infected individuals.

Hammond and Spanswick [153] demonstrated genomic DNA profiling by RAPD analysis in applications as wide ranging as paternity testing and forensic science, through investigation of genetic disease genes and identification of the sources of plant and animal products, to ecological population studies. PCR with random sequence primers offers a fast approach to genome profiling. The most popular technique of this type is called RAPD.

RAPD markers were reviewed by Bardakci [154]. Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. They emphasized that, the RAPD technique based on the PCR has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.

Ramalivhana et al. [155] examined the RAPD typing of clinical and environmental *Aeromonas hydrophila* strains from Limpopo Province, South Africa. The RAPD fingerprints obtained proved reproducible when repeated on three different occasions using whole cell DNA isolated from the *Aeromonas* strains. In total, 12 unique RAPD fingerprints were found. Costa et al. [156] performed RAPD profiling among *Candida albicans* isolates by using different primers. A total of 28 primers were screened, out of which, seven were selected because they presented reproducible DNA banding patterns for all the strains of *C. albicans*. The same genetic profile was obtained by RAPD method using OPG 14 (5'-GGATGAGACC-3') while the OPG 17 (5'- ACGACCGACA-3') demonstrated capacity to differentiate the isolates of *C. albicans* in genotypes. According to the results obtained, we concluded that by using these primers, the RAPD analysis may be useful in providing genotypic characteristics for *C. albicans* in epidemiological investigation.

Nuchprayoon et al. [157] examined RAPD for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. Comparative morphology and morphometry of microfilariae and a study of RAPD was performed. A phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). RAPD-PCR profiles showed specific bands characteristic for the Myanmar strain of *W. bancrofti*. The phylogenetic tree indicated two genetically distinct clusters of the Thai and Myanmar strains of *W. bancrofti*. The RAPD-PCR technique was suitable for differentiating Thai and Myanmar strains of *W. bancrofti*. The RAPD marker could be used for epidemiological assessment of the Myanmar strains of *W. bancrofti* in Thailand.

Sineo et al. [158] analysed the genetic markers by RAPD-PCR. RAPD-PCR is a new technique that, starting from genomic DNA allows, with the use of a single primer of "random" base composition to amplify a variable number of sequences that can give important informations if analyzed for linkage studies, gene mapping or phylogenetic purposes. In order to detect the possible application of this simple way of DNA-fingerprinting in individual identification and in cell lineages characterization they analyzed human and non-human primates DNA. When six different single primers of variable length were used, they resulted in individual or specific electrophoretic patterns with a better resolution using "short" primers. According to them, the individual electrophoretic patterns obtained by RAPD-PCR can be a simple and reliable approach to DNA analysis.

Pinto et al. [159] examined the patterns of genetic variation of samples of *Candida* spp. isolated from 37 HIV +ve patients in Vitoria, state of Espirito Santo, Brazil, having different infection episodes. These samples were subjected to RAPD analysis using 9 different primers. Reproducible and complex DNA banding patterns were obtained. Their experiments indicated evidence of dynamic process of yeast colonization in HIV infected patients, and also that certain primers were efficient in the identification of species of the *Candida* genus. The group concluded that RAPD analysis may be useful in providing genotypic characters for Candida species typing in epidemiological investigations, and also for the rapid identification of pathogenic fungi.

2.6.2 Blood DNA Analysis

Boriskin et al. [160] described the detection of HIV-l proviral DNA by PCR in clotted blood specimens from six hundred and ninety-two specimens from patients at high risk exposure to HIV. These samples were tested by a nested PCR procedure which had been optimized to give a sensitivity of detection of one copy of HIV-l proviral plasmid DNA, and the results were compared to those of testing for antibody to HIV on the same specimens. Direct sequencing of the PCR DNA products confirmed their specificity in all cases and demonstrated that no two patients gave the same predicted amino acid sequence for the V3 loop region. The sequences revealed both European/North American and African motifs at the crown of the V3 loop thus indicating a diversity of HIV strains in the Thames Region of South London. They emphasized that the confirmatory PCR for HIV-l can be carried out efficiently on the same clotted blood specimens as used for routine HIV serology on patients undergoing diagnostic evaluation.

2.7 Studies in Resource Poor Settings

Mwamburi et al. [161] developed a sustainable tool for clinical decision during HAART use. Understanding the total lymphocyte count (TLC) – CD4 count relationship could aide designing of predictive instruments for making clinical decisions during ART, especially in under-served resource poor settings. According to their study, TLC is simple and inexpensive and can be used in many ways to develop clinical decision-making tools in underserved resource-poor settings during HAART therapy. Badri et al. [162] discussed the utility of $CD4^+$ cell counts for early prediction of virological failure during ART in a resource limited setting. Viral load monitoring is not available for the vast majority of patients receiving ART in resource limited settings. In this study, they used a novel modeling approach that accounted for all $CD4^+$ cell count and viral load values measured during follow-up from the first date that viral load suppression was achieved.

Mehendale et al. [163] determined if the early immunological and virological events of HIV infection are unique in a setting with limited access to health care and HIV-1 subtype C infection. They undertook a prospective cohort study to characterize

the early natural history of HIV viral load and $CD4^+$ T lymphocyte counts in individuals with recent HIV sero conversion in India. Their data suggest that the more rapid HIV disease progression described in resource poor settings may be due to very early virological and host events following primary HIV infection. A rapid increase in viral load within the first 2 years after primary infection may have to be considered when applying treatment guidelines for ART and OI prophylaxis.

Ditangco et al. [164] did a retrospective/prospective cohort analytic study from May 1995 to May 1998 at the Research Institute for Tropical Medicine to determine the virologic, immunologic and clinical factors influencing the outcome (time to AIDS or death) of HIV disease among Filipino patients. Their study showed that patients who progressed to AIDS and/or did not survive during the follow up period had significantly higher viral load levels and lower $CD4^+$ T cell count. They suggest a similar threshold with the cut off values being adapted in the western countries and recommend adapting current international guidelines for initiating ART in the light that these guidelines are evolving into more conservative approach which is favorable for areas with limited resources.

Smith et al. [165] predicted the relationship between $CD4^+$ cell count nadirs and the toxicity profiles of ARV regimen. They suggested that a lower pre HAART $CD4^+$ count nadir may lead to a greater risk of experiencing HAART related toxicity and investigated the relationship between the pre-HAART $CD4^+$ count nadir, HAART and the occurrence of laboratory-defined toxicities. Assessment of circulating $CD4^+$ count change over time in HIV- infected subjects on ART is a central component of disease monitoring. The increasing number of HIV infected subjects starting therapy and the limited capacity to support $CD4^+$ count testing within resource limited settings have fueled interest in identifying correlates of $CD4^+$ count change such as total lymphocyte count, among others. The application of modeling techniques will be essential to this endeavor due to the typically nonlinear CD4 trajectory over time and the multiple input variables necessary for capturing CD4 variability.

Foulkes et al. [166] proposed a prediction-based classification approach that involves first stage modeling and subsequent classification based on clinically meaningful thresholds. Application of this method to an independent test sample

results in greater than 98% positive predictive value for $CD4^+$ count change. The prediction algorithm is derived based on a cohort of n = 270 HIV-1 infected individuals from the Royal Free Hospital, London who were followed for up to three years from initiation of ART. A test sample comprised of n = 72 individuals from Philadelphia and followed for a similar length of time is used for validation. The group suggested that their approach may be a useful tool for prioritizing limited laboratory resources for CD4 testing after subjects start ART.

Ghate et al. [167] gave a relationship between clinical conditions and $CD4^+$ counts in HIV-infected persons in Pune, Maharashtra, India. Their cross-sectional study was conducted among 137 HIV-infected persons investigated at an HIV reference centre in Pune. The study methods comprised pre-test counselling, informed consent, blood withdrawal and clinical evaluation. According to their study, absence of clinical conditions was found to be a good predictor of high $CD4^+$ counts. Sabin and Phillips [168] discussed whether HIV therapy should be started at a $CD4^+$ cell count above 350 cells/μl in asymptomatic HIV-1 infected patients. They reviewed the available data that contribute to the debate on the optimal time to initiate HAART in HIV-infected individuals with a $CD4^+$ cell count more than 350 cells/μl. They emphasized that the data would generally support initiation of HAART in patients with $CD4^+$ cell counts more than 350 cells/μl.

Balakrishnan et al. [169] evaluated the low cost monitoring of HIV infected individuals on HAART in developing countries. The standard methods to monitor HIV infection are flow cytometry based for $CD4^+$ T lymphocyte count and molecular assays to quantify plasma viral load of HIV. Few laboratories in resource limited countries can run these tests as a majority of the HIV infected individuals are poor. A number of currently available low-cost assays which require less expensive equipment and reagents include manual and ELISA based CD4 cell assays, and ultrasensitive reverse transcriptase quantitation (Cavidi) and p24 (ELAST) assays to monitor virus load. But better internal quality assurance and quality control (QA/QC) programmes are essential. Langford et al. [170] reviewed the predictors of disease progression in HIV infection. Host factors such as age, HLA and cytochrome P-450 (CYP) polymorphisms and psycho - social factors remain important, though often

unalterable, predictors of disease progression. Although gender and mode of transmission have a lesser role in disease progression, they may impact other markers such as viral load. They emphasized that readily measurable markers of disease such as total lymphocyte count, hemoglobin, body mass index and delayed type hypersensitivity may come into favour as ART becomes increasingly available in resource-limited parts of the world.

To estimate the probability of remaining free of AIDS for up to 25 years after infection with HIV by extrapolation of changes in $CD4^+$ lymphocyte count, Phillips et al. [171] designed a cohort study of subjects followed from time of HIV seroconversion until 1 January 1993 by using extrapolated linear regression slopes of $CD4^+$ count to predict development of AIDS after 1993. 44 men developed AIDS up to 1 January 1993. Their model predicted that 25% (95% confidence interval 16% to 34%/) would survive for 20 years after seroconversion and 18% (11% to 25/) for 25 years. Changing the $CD4^+$ count at which AIDS was assumed to occur did not alter the results. Younger patients had a higher chance of 20 year survival than older patients 32% (12% to 52%) for those aged <15, 26% (14/o to 38%) for those aged 15-29, and 15% (0%/6 to 31%/o) for those aged ≥ 30). These results suggest that even with currently available treatment up to a quarter of patients with HIV infection will survive for 20 years after seroconversion without developing AIDS.

To identify factors associated with sustained undetectable viraemia after HIV-1 seroconversion in treatment naive patients, and to describe concomitant $CD4^+$ cell count progression, Madec et al. [172] studied the spontaneous control of viral load and $CD4^+$ cell count progression among HIV-1 seroconverters. Seroconverters enrolled were assumed to control viraemia if at least two consecutive viral load measurements were < 400/500 copies/ml without treatment. Factors associated with undetectable viraemia were identified through a logistic regression. A joint model was used to describe simultaneously the $CD4^+$ cell count progression during and after that period and to identify factors associated with sustained undetectable viraemia. Of 2176 seroconverters, 145 (6.7%) spontaneously controlled viraemia. According to their study, women were more likely than men to achieve undetectable viraemia unlike patients who reported a symptomatic primary infection. AIDS and death rates

were significantly lower in patients achieving undetectable viraemia than in the others. The median period of undetectable viraemia was 11.2 months; on average, $CD4^+$ cell counts remained stable during that period, and decreased with a mean rate of 5 cells/ml per month thereafter. High $CD4^+$ cell count at the beginning of undetectable viraemia and non-symptomatic primary infection favoured the preservation of undetectable viraemia. A small proportion of seroconverters appeared to be able to control HIV viraemia spontaneously, mostly those without seroconversion illness and within a few years following seroconversion; this is associated with the benefits of slower $CD4^+$ cell count decline and improved long term prognosis. Such persons should be targeted for in depth investigation.

2.8 Informed consent

The Helsinki declaration is the reference statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. The main principle imposes to a physician to obtain an informed consent document from the research candidate before participating in a clinical research. However, this declaration did not take into account the socio-cultural context of developing countries where the majority of the patients are illiterate. Indeed, obtaining the consent of study participants is difficult and complex in resource poor settings especially in the context of HIV. Mallardi [173] reviewed the origin of informed consent. Ekouevi et al. [174] evaluated the procedure of obtaining informed consent from HIV-infected pregnant women at Abidjan, Cote d'Ivoire. The group studied the level of understanding of the informed consent document in the HIV-1 infected women enrolled in their project and conducted a cross-sectional survey within the DITRAME PLUS study in October and November 2001. A specific questionnaire was completed through interviews by social workers. After the interview, the consent document was checked to verify how it had been signed by the participants and countersigned by the research representative.

The role of disclosure in relation to assent to participate in HIV related research among HIV infected youth was assessed by Corneli et al. [175]. The objective of this study was to develop a culturally appropriate approach for obtaining assent from children aged eight to 17 years to participate in paediatric HIV related

operational research in Kinshasa, Democratic Republic of Congo. Included within this objective was to determine whether or not HIV disclosure should be included as part of the assent process prior to research participation, a component of research participation, or not incorporated in any aspect of the child's involvement in the research. They suggested that in settings, where most minors are unaware of their HIV infection, researchers should consider excluding the term, "HIV", when explaining HIV related research to minors, and omitting it from assent forms or informational sheets related to research participation. However, an individualized disclosure plan should be initiated with parents and caregivers at the time of enrolment in HIV related research, particularly in research that involves treatment.

Gray et al. [176] discussed the ethical dilemma of disclosure of HIV status on informed consent forms for protection of human subjects. According to them, the privacy of copies of consent forms provided to research participants cannot be guaranteed. Therefore, consent forms that disclose a subject's HIV status may result in breach of confidentiality and cause social harms. Under the ethical principle of beneficence defined in the Belmont Report, they recommend that disclosure of HIV status be through voluntary counseling and testing; however, whenever possible, copies of consent form should not specify HIV status.

Sastry et al. [177] examined the optimization of the HIV/AIDS informed consent process in India. In this study, they examined pregnant women's understanding of group education and counseling about HIV/AIDS provided within an antenatal clinic in Maharashtra, India. They found the use of visual aids during group counseling sessions increased women's overall understanding of key issues regarding informed consent from 38% to 72%. Moreover, if these same visuals were reinforced during individual counseling, improvements in women's overall comprehension rose to 96%. Their study demonstrates that complex constructs such as informed consent can be conveyed in populations with little education and within busy government hospital settings, and that the standard model may not be sufficient to ensure true informed consent.

2.9 Country Response

Rao et al. [178] examined the use of mathematical modeling in predicting the impact of the national response to HIV/AIDS epidemic in India, to assist the National AIDS Control Programme (NACP) - III planning team in determining appropriate targets to be activated during the project period (2007-2012). They constructed a dynamical model that captures the mixing patterns between susceptibles and infectives in both low risk and high risk groups in the population. Continuing the current level of interventions in NACP II, the model estimates 5.06 million people living with HIV/AIDS (PLHA) by the end of 2011. The current status of the epidemic appears to be less severe as compared to the trend observed in the late 1990s. They also predicted a positive role of implementation of ART of 90 percent of the eligible people in the country using convolution approaches.

Sehgal [179] reviewed the: time trends over a decade of HIV epidemic in Punjab, India, after the first AIDS patient in the northern state of Punjab was reported in May 1987. In this study, he show that the incidence in high-risk groups increased from 3 per 1000 in 1987 to 59 per 1000 in 1997, 73% of the cases being in the third and fourth decades of life, i.e. the most productive years. According to him, the intervention programme launched by the National AIDS Control Organization (NACO) appears to have had little impact on the epidemic. The researcher advocated the urgent need for more interactive programmes that include education concerning the modes of spread, course, financial implications and fatal outcome of the disease, instead of passive dissemination of information by posters and the media.

The NACP, launched in 1992, is being implemented as a comprehensive programme for prevention and control of HIV/AIDS in India [180]. Improved understanding of the complex HIV epidemic in India has enabled substantial changes to be made in the policy frameworks and approaches of NACP. The focus has shifted from raising awareness to behaviour change, from a national response to a more decentralised response and to increasing involvement of NGOs and network of PLHA. Phase-III (2007-2012) of NACP has the over all goal of halting and reversing the epidemic in India over the five year period. It has placed highest priority on

preventive efforts while, at the same time, seeking to integrate prevention with care, support and treatment through a four pronged strategy;

- Preventing new infections among high risk groups and general population through (a) saturation of coverage of high risk groups with targeted interventions; and (b) scaled up interventions in the general population.
- Providing greater care, support and treatment to larger number of PLHA.
- Strengthening the infrastructure, systems and human resources in prevention, care, support and treatment programmes at the district, state and national levels.
- Strengthening the nationwide strategic information management system.

Available evidence on HIV epidemic in India shows a stable trend at national level. Provisional estimates place the number of people living with HIV in India in 2008 at 22.7 lakhs with an estimated adult HIV prevalence of 0.29 percent. The epidemic is concentrated among high risk group populations and is heterogenous in its spread. The primary drivers of HIV epidemic in India are unprotected paid sex, unprotected sex between men and injecting drug use. Heterosexual route of transmission accounts for 87 percent of HIV cases detected.

2.10 Gaps in Existing Research

It can be seen from the above review that most of the investigation on HIV / AIDS is on the mode of HIV transmission, topographical distribution and expression. The biochemical analysis (with several metabolic tests simultaneously) of HIV +ve and AIDS affected human subjects along with their comparison with normal subjects have not been carried out in North Indian population. The association of $CD4^+$ cell count and different biochemical parameters by using correlation – regression analysis has not been developed in the targeted states of North India. Molecular analysis of HIV +ve human subjects with random primers to find out molecular similarity patterns between populations have not been undertaken in the targeted states of North India.

CHAPTER – 3

PURPOSE OF THE PRESENT STUDY

CHAPTER 3.0

Purpose of the Present Study

As per the Joint United Nations Programme on HIV and AIDS (UNAIDS), we are on the verge of a significant breakthrough in the AIDS response. The vision of a world with zero new HIV infections, zero discrimination, and zero AIDS-related deaths has captured the imagination of diverse partners, stakeholders and people living with and affected by HIV. At the end of 2010, an estimated 34 million people [31.6 million–35.2 million] were living with HIV worldwide, up 17% from 2001. Although the rate of HIV prevalence is substantially lower in Asia than in some other regions, the absolute size of the Asian population means it is the second largest group of people living with HIV. The prevalence of HIV among key populations at higher risk of infection – notably; female sex workers (FSW), injecting drug users (IDU) and men who have sex with men (MSM) – is high in several Asian countries; although over time, the virus is spreading to other populations also. The overall trends in this region hide important variations in the epidemics, both between and within countries. In many Asian countries, national epidemics are concentrated in relatively few localized regions.

Demographically the second largest country in the world, India has also the third largest number of people living with HIV/AIDS. As per the provisional HIV estimate of 2008-09, there are an estimated 22.7 lakh people living with HIV/AIDS in India. The HIV prevalence rate in the country is 0.29 percent (2008-09) and most infections occur through heterosexual route of transmission. However in the north-eastern region, injecting drug use is the major cause for the epidemic spread. The overall HIV prevalence among different population groups in 2008-09 continues to portray the concentrated epidemic in India, with a very high prevalence among high risk groups – IDU; 9.2%, MSM; 7.3%, FSW; 4.9% and sexually transmitted disease (STD) clinic attendees; 2.5% and low prevalence among antenatal clinic (ANC) attendees; 0.49%. Among IDUs, Chandigarh, Punjab, Delhi, Mumbai and Manipur have shown high levels of HIV prevalence. An overall decline in HIV prevalence among ANC attendees is noted at all India level and in high prevalence states.

However, rising trend among ANC attendees is observed in some low and moderate prevalence states such as Gujarat, Rajasthan, Orissa, Uttar Pradesh, Bihar and West Bengal.

India is considered to be a "next wave" country; that is, it stands at a critical point in its epidemic, with HIV poised to expand, but where large-scale prevention and other interventions today could help to contain a more serious epidemic in the future. As the second most populous nation in the world, even a small increase in India's HIV/AIDS prevalence rate would represent a significant component of the world's HIV/AIDS burden.

The present techniques and tests involved in HIV infection diagnosis are either costly or not easily accessible. The currently available diagnostic techniques; enzyme linked immunosorbent assay (ELISA), PCR analysis and $CD4^+$ cell count require highly skilled professionals and specialized equipments which are sparsely available in India. The cost involved in the test is also a considerable factor.

Keeping all these factors in view, we initially took this problem to develop correlation based on simple biochemical tests which can be performed any where in limited resource settings. Significant differences obtained (between normal and HIV +ve human subjects) in study could be used as basis for predicting HIV infection in target Indian population (North India). Further to study HIV infected population on the basis of phylogenetic analysis we perform molecular marker studies. This could be informative regarding differentiation of HIV infected population in Northern India and the results of primer amplification can work as reference data for further molecular analysis in future studies. Since the $CD4^+$ lymphocyte count is quite costly and the count facility is available at limited locations, an effort has been made to develop a simple empirical correlation based on regression analysis for estimation of $CD4^+$ lymphocyte population. This may decrease the analysis cost for HIV infection prediction in resource limited settings.

CHAPTER – 4

MATERIALS

&

METHODS

CHAPTER 4.0

Materials and Methods

This study was conducted at Department of Biotechnology, Meerut Institute of Engineering and Technology (MIET), Meerut. Due permission was taken from Institutional Research Board (IRB) and Institutional Ethical Committee (IEC) of MIET, for conducting the present investigation [Appendix- I, II]. The human blood samples of normal and HIV +ve subjects were taken from two locations. The $CD4^+$ cell count (estimated by flow cytometer) of HIV +ve subjects were provided by the respective medical centers. The analysis of various biochemical tests (Liver function, Kidney function, Lipid function and blood sugar) was conducted at an authorized medical diagnostic laboratory. Further, about 20% randomly selected sample from the above, were analyzed for molecular and phylogenetic analysis using random amplified polymorphic DNA (RAPD) – polymerase chain reaction (PCR) with random primers. The results obtained were subjected to statistical analysis to determine if there is any

- Presence of differential phylogenetic characteristics in HIV +ve human subjects at different locations in North India.
- Association between hepatic and renal profiles of human subjects with their HIV status at different locations in North India.
- Regional specificity of hepatic and renal profiles of HIV +ve human subjects.

4.1 Materials

4.1.1 Blood Samples

To complete the objectives of the present study, human blood samples were analysed.

Location: The probable locations for data and sample collection were first identified and then selected. The city was selected on the basis of its mixed rural/urban population structure; availability of HIV infected human subjects, categorisation of districts based on HIV Sentinel Surveillance 2004, 2005 and 2006 [181], literacy rate [182] and the percentage of floating population [183]. Blood

samples were obtained from Dayanand Medical College and Hospital, Ludhiana (Punjab; State-1) and Dr. S. N. Medical College, Jodhpur (Rajasthan; State-2) with due permission from the concerned authorities.

Originally, it was planned to obtain human blood samples of both normal and HIV +ve subjects from a minimum of four North Indian states. However, in Uttar Pradesh, the blood samples available were unspecified in terms of HIV and permanent resident status of the donor. The same was the case in Haryana. Therefore samples were collected from the medical centres at Punjab and Rajasthan with specified status. On the basis of clinical advice, the control group blood samples were collected from Rajasthan only, since the reference values from these samples could be valid from any of the North Indian states.

Sample Size: A total of 319 human blood samples were obtained during September, 2010 to June, 2011. Out of these 46 HIV +ve blood samples were collected from medical centre at Ludhiana (Punjab; State-1). A total of 102 HIV +ve, 53 AIDS affected and 118 age and sex matched normal blood samples were obtained from medical centre at Jodhpur (Rajasthan; State-2).

Sample Collection: 5 ml of the human blood sample per subject was obtained from the respective centres in EDTA and plain vacutainer tubes (2 ml and 3 ml respectively) for biochemical and molecular analysis. Since the samples include blood from known high-risk category, complete precautions were maintained for collection, storage and transportation of the same as per the national AIDS control organization (NACO) guidelines [184].

Sample Selection

Inclusion Criteria

- Blood samples from newly diagnosed HIV +ve persons (Untreated for HIV/AIDS through drugs).
- Blood samples from human subjects affected with AIDS (may or may not have undergone drug treatment for HIV/AIDS.

- Blood samples from normal human individuals not affected by diabetes or any of major heart, kidney or liver disease (and have not consumed drug for any major aliment in past one month).
- Native of Punjab or Rajasthan state.
- Age: 18 to 49 years.
- Sex: male, female.

Exclusion Criteria

- Blood samples from HIV +ve persons (not affected with AIDS) who have undergone drug treatment for HIV.
- Blood samples from normal human subjects affected by diabetes or any of major heart, kidney or liver disease (and have consumed drug for any major aliment in past one month).
- Non-native of Punjab or Rajasthan state.
- Unspecified blood samples (Normal or HIV +ve).
- Age: below 18 years and above 49 years.
- Sex: Trans-genders.

4.1.2 Reagents, Chemicals and Other Materials Used in the Study

All reagent kits used in biochemical tests (LFT, KFT, Lipid tests and glucose test) were of the AUTOPAK make from SIEMENS (Siemens Healthcare Diagnostics Ltd., Gujarat, India).

All chemicals and reagents used in DNA isolation from human blood were of analytical grade (HiMedia make) and include: Tris(hydroxymethyl)aminomethane hydochloride (Tris-HCl), Ethylenediaminetetraacetate (EDTA), potassium chloride (KCl), magnesium chloride ($MgCl_2$), Triton-X-100, sodium chloride (NaCl), sodium do-decyl sulphate (SDS), and sodium hydroxide (NaOH).

All chemicals, enzymes and buffers used in gel electrophoresis and random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR) were of analytical or molecular biology grade and include: agarose, Tris-HCl, borate, and EDTA [all HiMedia make], ethidium bromide; USB make, gel loading buffer (6X),

Taq DNA polymerase (3U/µl), Taq enzyme buffer; with $MgCl_2$ (10 X), dNTPs mix (10 mM), marker DNA [all Merck make].

Water, wherever used, was of double-distillation grade. EDTA-containing vacutainer tubes and plain vacutainer tubes used for collection of blood samples were of Hindustan syringes make. Rest all the chemicals were of analytical grade.

Buffers

- **Tris buffer-1** [10 mmol/L Tris-HCl, pH 8.0, 10 mmol/L KCl, 10 mmol/L $MgCl_2$, 2 mmol/L EDTA, pH 8.0 and 25 mL/L Triton X-100].
- **Tris buffer-2** [10 mmol/L Tris-HCl, pH 8.0, 10 mmol/L KCl, 10 mmol/L $MgCl_2$, 2 mmol/L EDTA, pH 8.0, 0.4 mol/L NaCl and 10 g/L sodium dodecyl sulfate].
- **Tris-EDTA (TE) buffer** [10 mmol/L Tris-HCl, pH 8.0 and 1 mmol/L EDTA].
- **Tris Borate EDTA (TBE) buffer 5X** [445 mmol/L Tris Base, 445 mmol/L Boric acid, and 10 mmol/L EDTA]

Primers

A total of 12 primers were used for the molecular marker studies. The first set consists of four RAPD primers as reported by Aikhionbare et al. [28]. The rest four sets (two primers each) were designed by using Primer3 and BLAST online tools available on National Center for Biotechnology Information (NCBI) web-site [29]. All primers were custom synthesized by Bioserve, Hyderabad.

4.1.3 Equipments Used

Bio-safety cabinet (Class-II, vertical)	[MAC make]
Biochemistry analyser	[Bayer RA-50]
Thermo cycler	[Corbett Palm cycler]
Electrophoresis gel system with Power supply	[Bangalore genei]
UV/Vis spectrophotometer	[Jasco V-530]
Gel documentation system	[Alpha Imager]
Cooling centrifuge	[MAC make]
Water bath	[MAC make]
Incubator	[MAC make]

Refrigerator (–80°C, -20°C & 4°C) [Dahian & Godrej make]

4.2 Methods

The following steps were performed to complete the objectives of the study:

I. Biochemical analysis of Liver [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, albumin, bilirubin total, and bilirubin direct], Kidney (urea and creatinine) Lipid (cholesterol and triglyceride) and Sugar parameters.

II. Non Linear Regression Analysis

III. Whole Blood DNA Extraction.

IV. Molecular Profiling.

V. Phylogenetic analysis through UPGMA based Jacquard's similarity coefficient.

VI. Analysis of Molecular Variance (AMOVA)

4.2.1 Methods for Biochemical Analysis

The biochemical testing was performed at YPL Diagnostics, Meerut, under the supervision of a qualified medical pathologist. All the analysis was performed in triplicate by using the pre-set software programme. The laboratory is highly equipped and uses standardized operation protocols for various tests. The disposal of wastes was done in a standardized manner through contract with a certified waste management company.

4.2.1.1 Liver Function Tests

4.2.1.1.1 Aspartate aminotransferase

It involves *in-vitro* test for the quantitative determination of aspartate aminotransferase (AST) in human serum and plasma. The International Federation of Clinical Chemistry (IFCC) recommended in 1977 and 1980 standardized procedures for AST determination [185], including optimization of substrate concentrations, employment of Tris(hydroxymethyl)-aminomethane buffers, pre-incubation of combined buffer and serum to allow side reactions with nicotinamide adenine

dinucleotide (NADH; reduced) to occur, substrate start, and optional pyridoxal phosphate activation.

2-oxoglutarate + L-aspartate ⟶ L-glutamate + oxalacetate

AST is the enzyme which catalyzes this equilibrium reaction. The oxalacetate increase is measured in a subsequent indicator reaction which is catalyzed by malate dehydrogenase (MDH).

oxalacetate + NADH + H^+ ⟶ L-malate + NAD^+

In the second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (Measured photometrically) is directly proportional to the rate of formation of oxalacetate, and thus the AST activity.

4.2.1.1.2 Alanine aminotransferase

It involves *in-vitro* test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma. In 1977 and 1980, the IFCC recommended standardized methods for the determination of ALT [186] with optimized substrate concentrations, use of Tris buffer, simultaneous pre-incubation of serum with buffer (to avoid competing reactions with NADH), substrate start, and pyridoxal phosphate activation.

2-oxoglutarate + L-alanine ⟶ L-glutamate + Pyruvate

ALT is the enzyme which catalyzes this equilibrium reaction. The pyruvate increase is measured in a subsequent indicator reaction which is catalyzed by lactate dehydrogenase (LDH).

Pyruvate + NADH + H^+ ⟶ L-lactate + NAD^+

In the second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate of formation of pyruvate, and thus the ALT activity.

4.2.1.1.3 Alkaline Phosphatase

It involves *in-vitro* test for the quantitative determination of alkaline phosphatase (ALP) in human serum and plasma. Alkaline phosphatase in serum

consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from germ cells.

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-nitrophenol.

p-Nitrophenylphosphate + H_2O ⟶ Phosphate + p-Nitrophenol

In this process AMP serves as transient phosphate acceptor. The release of coloured p-nitrophenol is proportional to the ALP activity and can be measured photometrically [187].

4.2.1.1.4 Albumin

It involves *in-vitro* test for the quantitative determination of albumin in human serum and plasma. Albumin is a carbohydrate-free protein, which constitutes 55-65% of total plasma protein. The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromocresol green, an anionic dyestuff, to form a blue-green complex.

Albumin + Bromocresol green ⟶ Albumin – Bromocresol green (Complex)

The color intensity of the blue-green color is directly proportional to the albumin concentration and can be determined photometrically [188].

4.2.1.1.5 Bilirubin total and Bilirubin direct

It involves quantitative determination of direct bilirubin and total bilirubin in serum, heparinized plasma or EDTA plasma by the Jendrassik-Grof method. Distinguishing between direct and indirect bilirubin is a valuable aid in the differential diagnosis of different forms of jaundice. A direct bilirubin value of < 20 % total bilirubin is an indicator of jaundice of pre-hepatic origin. This value can increase to > 50 % in hepatic and post-hepatic jaundice. Jendrassik-Grof method - In the presence of caffeine accelerator, total bilirubin couples with sulfanilic acid to form a red

azobilirubin dye, the color intensity of which is proportional to the bilirubin concentration [189].

Bilirubin-total + Sulfanilic acid ⟶ Bilirubin-total–sulfanilic acid (Azobilirubin dye complex)

Determination of direct bilirubin is performed without caffeine additive.

4.2.1.2 Kidney Function Tests

The kidney function tests employ blood samples for estimation of urea and creatinine. The human body's homeostatic mechanism maintains the concentration of various metabolites at a steady state in the blood whereas their concentration changes continuously in the urine.

4.2.1.2 .1 Urea

It employs an enzymatic in vitro test for the quantitative determination of urea in human serum, plasma and urine. Urea is hydrolysed in presence of urease to produce ammonia and CO_2.

$$\text{Urea} + H_2O + 2\,H^+ \longrightarrow 2\,NH_4^+ + CO_2$$

The ammonia produced combines with 2 – oxoglutarate and NADH in presence of glutamate dehydogenase (GLDH) to yield glutamate and NAD.

$$2\,NH_4^+ + 2\text{ - Oxoglutarate} + 2\,NADH \longrightarrow H_2O + 2\,NAD^+ + \text{Glutamate}$$

The decrease in absorbance due to consumption of NADH is measured kinetically [190].

4.2.1.2.2 Creatinine

It employs kinetic in vitro assay using rate-blanking and compensation for the quantitative determination of creatinine in human serum, plasma and urine. In alkaline solution, creatinine forms a yellow-orange complex with picrate.

creatinine + picric acid ⟶ creatinine – picric acid complex

The color intensity is directly proportional to the creatinine concentration and can be measured photometrically [191].

4.2.1.3 Lipid Function Tests

4.2.1.3.1 Total cholesterol

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. This method is based on the determination of Δ4 cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement of hydrogen peroxide formed by the Trinder reaction. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary standards. Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. Cholesterol esters are cleaved by the action or choesterol esterase to yield free choesterol and fatty acids.

Cholesterin ester + H_2O ⟶ Cholesterol + fatty acids

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en 3-one and hydrogen peroxide.

Cholesterol + O_2 ⟶ 4 Cholesten - 3 - one + H_2O_2

Hydrogen peroxide thus created forms a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase.

2 H_2O_2 + Phenol + 4 - Aminoantipyrine ⟶ quinoeimine dye + 4 H_2O

The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically [192].

4.2.1.3.2 Triglyceride

Triglycerides are esters of the trihydric alcohol glycerol with three long chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The method described here utilizes a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol.

Triglycerides + 3 H_2O ⟶ Glycerol + 3 RCOOH

This was followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide.

Glycerol + ATP ⟶ G-3-P + ADP

G-3-P + O_2 ⟶ DAP + H_2O_2

H_2O_2 + 4-aminoantipyrine + p-chlorophenol → 4-(p-benzoquinone-monoimino)-phenazone + 2 H_2O + HCL

The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction) [193].

4.2.1.4 Blood Glucose

Glucose is the central energy source of the cells in the organism. The most common supply follows hydrolytic cleavage of polymeric carbohydrates, in general starch. Glucose is a monosaccharide with a postprandial concentration of 5 mmol/l in the blood and serves as an indispensable energy-supply for cellular functions. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation. The test is based on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in hydrogen peroxide, which is subsequently used for the generation of a coloured product by peroxidase.

Glucose + O_2 + H_2O ⟶ Gluconic acid + H_2O_2

2 H_2O_2 + Phenol + 4-Amino-antipyrine ⟶ Red Chinonimin + 4 H_2O

In the Trinder method the carcinogenic ortho-dianisidine used in earlier formulations has been replaced by phenol and 4-amino-antipyrine [194].

4.2.2 Non Linear Regression Analysis (Empirical correlation) [166]

To predict the $CD4^+$ cell count by using biochemical parameters, a non linear regression equation was developed using sigma plot software. A general non linear regression equation for correlation between $CD4^+$ cell count and any two biochemical parameter can be developed as follows:

$f = a * x^b * y^c$

Where f = Predicted $CD4^+$ cell count,

a, b and c are constants to be developed using non linear regression analysis, and

x and y = the biochemical parameters, with which the correlation is being developed.

4.2.3 Whole Blood DNA Extraction

This method used standard analytical grade chemicals available in the Department of Biotechnology, MIET, Meerut.

- 0.154 M Sodium chloride (Normal Saline): 9 gm of NaCl was dissolved in 1 liter of distilled water. The solution was autoclaved at 15 p.s.i. for 20 minutes.
- 5 M Sodium chloride: 292.2 gm of NaCl was dissolved in 1 liter of distilled water. The solution was autoclaved at 15 p.s.i. for 20 minutes.
- Tris Buffer – I was diluted up to a total volume of 1 liter with distilled water and autoclaved at 15 p.s.i. for 20 minutes.
- Tris Buffer – II was diluted up to a total volume of 1 liter with distilled water and autoclaved at 15 p.s.i. for 20 minutes.
- Tris – EDTA Buffer was diluted up to a total volume of 1 liter with distilled water and autoclaved at 15 p.s.i. for 20 minutes.
- Ethanol (100%) pre-chilled to -20°C.

Protocol for DNA Extraction

The protocol for DNA extraction was modified from the procedure given by Hirata et al. [195].

- 400 µl of blood was taken into a 2 ml eppendorf tube and 600 µl of 0.154 M NaCl (normal saline) was added to it.
- The contents were mixed (homogenized) by gently inverting the tube for 30 seconds and then centrifuged for 5 minutes at 4300 rpm (1760 g).
- The supernatant was discarded and to the pellet, 1 ml of Tris Buffer – I was added. [The pellet was broken by vortexing and rinsed well in Tris Buffer – I, so as to clean the white blood cells from residual of hemoglobin]
- The eppendorf tube was incubated at room temperature (RT) for 5 minutes and then centrifuged at 5500 rpm (2880 g) for 8 minutes.
- The supernatant was discarded and the pellet was washed twice with Tris Buffer – I.
- After second washing with Tris Buffer – I and centrifugation, the supernatant was removed.
- To the left over pellet, 220 µl of Tris Buffer – II was added and then the eppendorf tube was incubated in water bath at 56°C for 15 minutes.
- After incubation, 100 µl of 5 M NaCl was added (and mixed) to precipitate the cellular proteins.
- The eppendorf tube was then centrifuged at 4000 rpm (1523 g) for 10 minutes.
- The supernatant was carefully taken in a fresh 1.5 ml eppendorf tube.
- Double volume of cold (-20°C) absolute ethanol was added and the contents were mixed by inverting eppendorf tube to facilitate DNA precipitation.
- The eppendorf tube was then incubated at 4°C for 15 minutes.
- After incubation, the tube was centrifuged at 5500 (2880 g) rpm for 3-4 minutes.
- The supernatant was discarded carefully and the eppendorf tube was then air dried for 10 minutes.
- To the pellet, 100 µl of Tris – EDTA Buffer was added and the DNA samples were then stored at 4°C for further analysis (or at – 20°C for later uses).

As per the standardized protocol, the yield of DNA in this procedure was 100 to 300 ng/µl. The DNA isolated from the HIV infected human blood samples was then quantified at ultra violet absorbance of 260 nm using UV-VIS scanning spectrophotometer (Jasco). Reference was set against Tris – EDTA Buffer and then after thorough rinsing of quartz cuvette, the absorbance of the sample was measured at 260 nm and 280 nm. The ratio of optical density (OD): OD260 / OD 280 provide an estimate of purity of nucleic acid. Pure preparation of DNA has the ratio of 1.8 - 2.0 and the concentration in µg/ml calculated as 1 OD at 260 nm is equivalent to 50 µg/ml of double stranded DNA [196].

4.2.4 Molecular Profiling

The blood genomic DNA was amplified by PCR using thermal cycler. Primers used for PCR reaction are segments of DNA which amplify corresponding region by randomly binding to the genome. A form of PCR; RAPD uses several arbitrary, short primers (10-14 nucleotides), which are annealed with the template of genomic DNA [28]. In theory, the primer anneals with many regions of the genome simultaneously. However, geometric amplification only occurs in those regions in which the 3' end of the annealed primers face one another on opposite strands and are no more than 3 Kb apart. Thus, the technique essentially scans a genome for these small inverted repeats and amplifies intervening DNA sequences of variable length. By resolving the resulting patterns, a profile was generated from a random PCR reaction. Consistency of band profiles was assessed by triplicate analysis. The DNA segment thus amplified were visualised by agarose gel electrophoresis. The protocol parameters for PCR analysis [28] were standardized for optimal results and are shown in different tables below.

4.2.4.1 PCR Reaction for RAPD

Based on amount of DNA, PCR reaction was setup with each individual primer. DNA amplification for RAPD was performed in a total volume of 25 µl. Components as mentioned in table 4.1 were mixed gently in 0.50 µl thin walled PCR-tubes.

Table 4.1 PCR reaction mixture

S. No.	Components for PCR reaction	Volume (µl)
1.	Water (double-distilled, de-ionised)	14.5
2.	Taq Assay Buffer (10 X)	2.5
3.	dNTPs Mix (10mM)	1.5
4.	DNA (25ng/ul)	2.0
5.	Primer (25 ng/µl)	4.0
6.	Taq DNA Polymerase (3U/µl)	0.5
7.	Total Volume	25

The PCR tubes were transferred to the DNA thermal cycler (Corbett). Amplification reactions were performed with the thermal profile as mentioned in table 4.2.

Table 4.2 Thermal profile for RAPD-PCR reactions

S. No.	Steps		Temperature (^{o}C)	Duration	Number of cycles
1.	Initial Denaturation		94	4 minutes	1 repeat
2.	a	Denaturation	94	30 seconds	40 Repeats
	b	Annealing	PSAT*	30 seconds	
	c	Extension	72	1 minute	
3.	Final Extension		72	3 minutes	1 repeat
4.	Hold		4	Till the reaction is stopped	

*PSAT - Primer specific annealing temperature

The molecular characteristics and primer specific annealing temperature (PSAT) for each primer is given in table 4.3. These PSATs were obtained by running a gradient PCR reaction in different temperature ranges for different primers with sample DNA. It can be seen from table 4.3 that most primers selected were GC rich which are known to generate high amplification [28].

Table 4.3 Molecular Characteristics of Primers and their Specific Annealing Temperature

S. No.	Primer Code	Sequence (5'→3')	Molecular Weight (in Daltons)	Temperature Range (°C)	PSAT (°C)
1.	FA-1	GGTGCACGTT	3059	28 – 39	31
2.	FA-2	GTTTCGCTCC	2970	28 – 39	28
3.	FA-3	CCACGGGAAG	3062	28 – 39	31
4.	FA-4	ACGGCGTATG	3068	28 – 39	33
5.	AST-1	CGGACCCCCGCAAG	4219	52 – 63	52
6.	AST-2	CGCGGGTTCGCAGT	4296	52 – 63	52
7.	ALT-1	TACGCCGGGCAGCA	4274	52 – 63	59
8.	ALT-2	CGCGGGTTCGCAGT	4296	52 – 63	53
9.	ALP-1	CCCGCCGTGGGTCT	4232	52 – 63	55
10.	ALP-2	ACGGCGGGGAGGAC	4379	52 – 63	53
11.	ALB-1	GCAGCGGCACAGCA	4283	52 – 63	62
12.	ALB-2	TGCAGCGGCACAGC	4274	52 – 63	61

PSAT – Primer Specific Annealing Temperature

4.2.4.2 Agarose Gel Electrophoresis

The agarose gel electrophoresis was adapted from the protocol as given by Johnson and Grossman [197]. A 4% agarose (LMP) gel was casted in the gel tray after dissolving the agarose in TBE buffer (ethidium bromide dye at a final concentration of 0.5 µg/ml was added to the molten gel at around 55-60 °C). 5 µl of gel loading buffer (6 X) was added to each PCR vial after the completion of the reaction. 15 µl of RAPD-PCR product from each PCR vial was loaded separately in each individual well in gel (total 44 samples with one primer set). The corner lanes were loaded with 10 µl of the readymade marker DNA (100 bp DNA ladder). The products were run in the agarose gel at 50 volts.

The gel was then observed under UV light in a trans-illuminator (Alpha imager) and its image was saved with the help of the gel documentation system. Bands were sized and matched directly on the gel images and a 0-1 matrix sheet was prepared on the basis of presence and absence of bands. The data was converted into dendogram form (phylogenetic tree) by Numerical Taxonomy System (NTSYS)

software and analysed using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA).

4.2.5 Phylogenetic analysis

Phylogenetic analysis was performed through UPGMA based analysis [198]. The UPGMA is one of the simplest methods of tree construction. It was originally developed for constructing taxonomic phenograms, i.e. trees that reflect the phenotypic similarities between operational taxonomic units (OTUs), but it can also be used to construct phylogenetic trees if the rates of evolution are approximately constant among the different lineages. UPGMA employs a sequential clustering algorithm, in which local topological relationships are identified in order of similarity, and the phylogenetic tree is built in a stepwise manner. We first identify from among all the OTUs the two OTUs that are most similar to each other and then treat these as a new single OTU. Such an OTU is referred to as a composite OTU. Subsequently from among the new group of OTUs we identify the pair with the highest similarity, and so on, until we are left with only two OTUs.

4.2.5.1 Analysis of Genetic Diversity

Polymorphic products from RAPD-PCR assay were calculated qualitatively for presence (1) or absence (0) of bands. The proportion of bands that have been shared between any of the two individuals screened (averaged over loci RAPD primers) were used as the measure of similarity.

Genetic diversity or Marker index was calculated using the following formula [199]:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

P_{ij} is the frequency of the j^{th} allele for marker, i and the summation extends over n alleles. The calculation was based on the number of bands in RAPD.

4.2.5.2 Calculation of Resolving Power [200]

Resolving Power is based on the distribution of alleles within the sampled genotypes. The most efficient means of separating any group of taxa for identification

purpose is to progressively divide the group into equal subgroups. It follows that the value of a primer/technique will be a function of how many band positions are generated and of how close to the optimal condition (division of taxa into equal halves) each band position is. The value of a particular band position can be measured most simply then by its similarity to the optimal condition (50% of genotypes containing the band). This "band informativeness" (I_b) can be represented into a 0-1 scale by the simple formula:

$$\mathbf{I_b = 1-(2 \times (0.5 - P_{ij}))}$$

where P_{ij} is the proportion of the 44 genotypes containing the band.

The I_b value was calculated for all 44 informative RAPD bands that were scored in the study. If all bands were optimally informative, then the most useful primer would be simply those that generated the most band positions. Given that bands can be weighted according to their similarity to optimal informativeness, the ability of a primer or technique to distinguish between large numbers of genotypes could be represented by the sum of these adjusted values. This can be described as the Resolving Power of the primer (Rp), where:

$$\mathbf{Rp = \sum I_b}$$

4.2.6 Analysis of Molecular Variance (AMOVA)

To describe the partitioning of genetic variation between and within groups, Analysis of Molecular Variance (AMOVA) can be used. AMOVA is a method of estimating population differentiation directly from molecular data and testing hypotheses about such differentiation. The significance of the covariance components associated with the different possible levels of genetic structure (among individuals, among populations, among groups) is tested using non-parametric permutation procedures given by Excoffier et al. [142]. For an AMOVA, a distance matrix is created within Arlequin (software programme) or included within the input file. Arlequin partitions the sum of squared deviations from distance matrix into hierarchical variance components, which are tested for significance using permutation tests. Mean square (MS) is sum of squares (SS) divided by its DF. The value of F is the MS among populations divided by MS within population.

CHAPTER – 5

RESULTS & DISCUSSION

CHAPTER 5.0

Results and Discussion

In the present investigation, blood samples from HIV +ve human subjects from two topographically distinct locations; Punjab (State – 1) and Rajasthan (State – 2) from Northern India region have been taken for comparison. The analysis of blood samples from HIV +ve and normal human subjects was divided into

I. Biochemical analysis
II. CD 4^+ T Lymphocyte Analysis (including Non Linear Regression Analysis)
III. Molecular marker analysis (including Phylogenetic analysis and AMOVA)

The parameters of biochemical analysis were

- Liver Function Tests (LFTs) which included estimation of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, serum albumin, bilirubin total and bilirubin direct.
- Kidney Function Tests (KFTs) which included estimation of serum creatinine and blood urea.
- Lipid Function Tests which included estimation of total serum cholesterol and serum triglyceride.
- Random blood glucose test

The results of the biochemical analysis were used to develop correlation and regression based relationships between $CD4^+$ cell count and other parameters under study. In the molecular marker analysis, amplification with random primers was performed for assessing phylogenetic relationship between subjects of two populations and also to determine possible primer sequences for HIV differentiation.

The results of all the analysis is sequentially recorded in the following pages with appropriate discussion.

5.1 Biochemical Analysis

The results of the different biochemical parameters used in the present study were compared using the statistical tool-student's *t*-test for comparison amongst different groups of normal, HIV and AIDS subjects in different states. The result (*t*-value) is reported as either significant or in-significant depending upon their resultant comparison with *t* - critical value.

5.1.1 Liver Function Analysis

5.1.1.1 State – 1 (Punjab) Liver Function Analysis in 18-49 years age group

Table 5.1 shows the mean concentration and *t*-test analysis of Liver Function Tests (LFTs) in Normal and HIV subjects of 18-49 years age group from state-1 (Punjab).

Table 5.1 Mean concentration and *t*-test analysis of Liver Function in Normal and HIV subjects from State-1 (Punjab)

Liver Function Tests [State-1 (Punjab)] [Age Group: 18-49 years]					
S. No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		Normal (n=118)	**HIV** (n=46)		
1.	Aspartate aminotransferase	23.03 ± 8.37 IU/L	33.50 ± 7.27 IU/L	-7.94	Significant***
2.	Alanine aminotransferase	19.58 ± 8.25 IU/L	28.83 ± 5.45 IU/L	-8.36	Significant***
3.	Alkaline phosphatase	143.06 ± 30.48 IU/L	197.11 ± 63.28 IU/L	-5.55	Significant***
4.	Serum albumin	3.63 ± 0.56 g/dl	2.81 ± 0.40 g/dl	10.43	Significant***
5.	Bilirubin total	0.46 ± 0.13 mg/dl	0.60 ± 0.33 mg/dl	-2.74	Significant**
6.	Bilirubin direct	0.24 ± 0.05 mg/dl	0.34 ± 0.25 mg/dl	-2.74	Significant**

**** p < 0.01, *** p < 0.001**

The mean concentration of enzyme aspartate aminotransferase (AST) for HIV subjects, 33.50 ± 7.27 IU/L of age 18-49 years in state-1 was much higher as compared to the normal subjects, 23.03 ± 8.37 IU/L within the same state. The *t*-test for enzyme AST shows highly significant *t*-value of -7.94 ($p < 0.001$) between the normal and HIV groups of age 18-49 years in state-1. Similarly, the mean concentration values for enzymes - alanine aminotransferase (ALT); 28.83 ± 5.45 IU/L and alkaline phosphatase (ALP); 197.11 ± 63.28 IU/L in HIV subjects in state-1 (Punjab), were significantly higher as compared to the ALT (19.58 ± 8.25 IU/L) and ALP (143.06 ± 30.48 IU/L) values respectively in normal subjects with in the same state. The *t*-test for enzymes; ALT and ALP shows significant *t*-values of -8.36 ($p < 0.001$) and -5.55 ($p < 0.001$) respectively between normal and HIV subjects within the same state

The mean concentration of serum protein albumin (ALB) in HIV subjects, 2.81 ± 0.40 g/dl was lower as compared to the normal subjects, 3.63 ± 0.56 g/dl in state-1 (Punjab). The *t*-test for serum albumin shows highly significant results with *t*-value of 10.43 ($p < 0.001$) between normal and HIV subjects within the same state.

The mean concentration values for Bilirubin-Total (Bil-T); 0.60 ± 0.33 mg/dl and Bilirubin-Direct (Bil-D); 0.34 ± 0.25 mg/dl in HIV subjects in state-1 (Punjab) were significantly higher as compared to the Bil-T (0.46 ± 0.13 mg/dl) and Bil-D (0.24 ± 0.05 mg/dl) respectively in normal subjects within the same state. The increase in Bil-T and Bil-D was significant with *t*-value of -2.74 ($p < 0.01$) each for normal and HIV subjects in state-1 (Punjab).

5.1.1.2 State – 2 (Rajasthan) Liver Function Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of LFTs in Normal and HIV subjects of 18-49 years age group from state-2 (Rajasthan) are shown in table 5.2. The mean concentration for enzyme AST in HIV subjects (34.31 ± 6.45 IU/L) of 18-49 years age in state-2 was higher as compared to the normal subjects (23.03 ± 8.37 IU/L) within the same state. The *t*-test for enzyme AST shows highly significant *t*-value of -11.28 ($p < 0.001$) between normal and HIV groups of the same state.

Similarly, the mean concentration for enzymes; ALT (29.54 ± 5.28 IU/L) and ALP (189.80 ± 48.51 IU/L) in HIV group of 18-49 years age in state-2 were significantly higher as compared to the concentration of ALT (19.58 ± 8.25 IU/L) and ALP (143.06 ± 30.48 IU/L) respectively in normal subjects of the same state. The *t*-test for enzymes; ALT and ALP shows highly significant *t*-values of -10.80 ($p < 0.001$) and -8.40 ($p < 0.001$) respectively between normal and HIV subjects in state-2. The mean concentration of serum ALB for HIV subjects, 2.80 ± 0.38 g/dl was significantly lower as compared to the normal subjects, 3.63 ± 0.56 g/dl in state 2 (Rajasthan). The *t*-test for ALB was highly significant with *t*-value of 13.04 ($p < 0.001$) between normal and HIV subjects within the same state.

Table 5.2 Mean concentration and *t*-test analysis of Liver Function in Normal and HIV subjects from State-2 (Rajasthan)

Liver Function Tests [State-2 (Rajasthan)] [Age Group: 18-49 years]					
S. No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		Normal (n=118)	**HIV** (n=102)		
1.	Aspartate aminotransferase	23.03 ± 8.37 IU/L	34.31 ± 6.45 IU/L	-11.28	Significant***
2.	Alanine aminotransferase	19.58 ± 8.25 IU/L	29.54 ± 5.28 IU/L	-10.80	Significant***
3.	Alkaline phosphatase	143.06 ± 30.48 IU/L	189.80 ± 48.51 IU/L	-8.40	Significant***
4.	Serum albumin	3.63 ± 0.56 g/dl	2.80 ± 0.38 g/dl	13.04	Significant***
5.	Bilirubin total	0.46 ± 0.13 mg/dl	0.53 ± 0.31 mg/dl	-2.23	Significant*
6.	Bilirubin direct	0.24 ± 0.05 mg/dl	0.27 ± 0.19 mg/dl	-1.70	In-significant

*** p < 0.05, *** p < 0.001**

The mean concentration of Bil-T (0.53 ± 0.31 mg/dl) in HIV subjects was higher as compared to the Bil-T (0.46 ± 0.13 mg/dl) in normal subjects in state-2. The *t*-test for Bil-T shows significant t-value of -2.23 ($p < 0.05$) between normal and HIV subjects within the same state. The mean concentration of Bil-D (0.27 ± 0.19 mg/dl) in HIV subjects was slightly higher as compared to the Bil-D (0.24 ± 0.05 mg/dl) in

normal subjects from state-2. The increase in Bil-D was in-significant with a *t*-value of -1.70 between normal and HIV subjects within the same state.

5.1.1.3 Liver Function Comparison between HIV Groups in State-1 and State-2

The comparison of the *t*-values of normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan) for analysis of liver function tests is shown in table 5.3. The *t*-value comparison of normal versus HIV subjects for all parameters in LFTs (AST, ALT, ALP, ALB, Bil-T and Bil-D) shows significant result in state-1. State-2 also shows similar significant results of *t*-values except for Bilirubin direct, which were in-significant, while comparing normal and HIV subjects. The results of *t*-test for AST, ALT, ALP, and ALB were more significant and hence higher *t*-values in state-2 as compared to state-1. This trend was however reversed in case of last two parameters of LFTs- Bil-T and Bil-D, where the *t*-values were lower in state-2 as compared to state-1.

Table 5.3 Comparison of *t*-values of Liver Function for Normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan)

Liver Function Tests			
S. No.	**Tests**	***t*- value**	
		State-1	**State-2**
1.	Aspartate aminotransferase	-7.94 (Significant)	-11.28 (Significant)
2.	Alanine aminotransferase	-8.36 (Significant)	-10.80 (Significant)
3.	Alkaline phosphatase	-5.55 (Significant)	-8.40 (Significant)
4.	Serum albumin	10.43 (Significant)	13.04 (Significant)
5.	Bilirubin total	-2.74 (Significant)	-2.23 (Significant)
6.	Bilirubin direct	**-2.74 (Significant)**	-1.70 (In-significant)

The mean concentration and *t*-test analysis of liver function tests in HIV subjects of state-1 (Punjab) versus state-2 (Rajasthan) are shown in table 5.4. While comparing HIV subjects in state-1 and state-2, all LFTs (AST, ALT, ALP, ALB, Bil-T and Bil-D) show in-significant results in terms of their mean concentration and *t*-values. This reveals that there is no difference or very in-significant difference between the various concentrations of LFTs in HIV subjects of the two states.

Table 5.4 Mean concentration and *t*-test analysis of Liver Function Comparison in HIV subjects of State-1 (Punjab) versus State-2 (Rajasthan)

Liver Function Tests [State-1 versus State-2]					
S. No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		HIV (n=46)	**HIV** (n=102)		
1.	Aspartate aminotransferase	33.50 ± 7.27 IU/L	34.31 ± 6.45 IU/L	-0.65	In-significant
2.	Alanine aminotransferase	28.83 ± 5.45 IU/L	29.54 ± 5.28 IU/L	-0.74	In-significant
3.	Alkaline phosphatase	197.11 ± 63.28 IU/L	189.80 ± 48.51 IU/L	-0.70	In-significant
4.	Serum albumin	2.81 ± 0.40 g/dl	2.80 ± 0.38 g/dl	0.20	In-significant
5.	Bilirubin total	0.60 ± 0.33 mg/dl	0.53 ± 0.31 mg/dl	1.14	In-significant
6.	Bilirubin direct	0.34 ± 0.25 mg/dl	0.27 ± 0.19 mg/dl	1.66	In-significant

5.1.1.4 Age Group Analysis of Liver Function

To analyze the overall age based liver function, the data from the two states was combined and the total samples were divided into different age groups of normal, HIV and AIDS as shown in table 5.5.

Table 5.5 Normal, HIV & AIDS Subjects among different age groups

S. No.	Name of Groups	Age Groups	Total Number (n)		
			Male (M)	Female (F)	Total (M + F)
1.	Normal (N_{18-29})	18-29 years	14	23	37
2.	Normal (N_{30-39})	30-39 years	25	20	45
3.	Normal (N_{40-49})	40-49 years	20	16	36
4.	HIV (H_{18-29})	18-29 years	25	23	48
5.	HIV (H_{30-39})	30-39 years	31	24	55
6.	HIV (H_{40-49})	40-49 years	24	21	45
7.	HIV (H_{18-49})	18-49 years	80	68	148
8.	AIDS (A_{18-49})	18-49 years	33	20	53

5.1.1.5 Liver Function Analysis in 18-29 years age group

Table 5.6 shows the mean concentration and *t*-test analysis of LFTs in Normal and HIV subjects of 18-29 years age group. The mean concentration of enzyme aspartate aminotransferase (AST) in HIV subjects (33.88 ± 6.35 IU/L) was much higher as compared to the normal subjects (18.97 ± 4.48 IU/L) of 18-29 years age group. The *t*-test for enzyme AST shows highly significant *t*-value of -12.68 ($p < 0.001$) between normal (control) and HIV subjects of 18-29 years age group. Thus AST activity was significantly elevated by HIV.

Table 5.6 Mean concentration and *t*-test analysis of Liver Function in Normal and HIV subjects [Age Group: 18-29 years]

Liver Function Tests [Age Group: 18-29 years]					
S. No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		Normal (n=37)	**HIV** (n=48)		
1.	Aspartate aminotransferase	18.97 ± 4.48 IU/L	33.88 ± 6.35 IU/L	-12.68	Significant***
2.	Alanine aminotransferase	17.65 ± 6.55 IU/L	28.81 ± 5.63 IU/L	-8.27	Significant***
3.	Alkaline phosphatase	147.59 ± 23.37 IU/L	211.25 ± 42.31 IU/L	-8.82	Significant***
4.	Serum albumin	3.51 ± 0.52 g/dl	2.73 ± 0.39 g/dl	7.67	Significant***
5.	Bilirubin total	0.41 ± 0.15 mg/dl	0.49 ± 0.36 mg/dl	-1.47	In-significant
6.	Bilirubin direct	0.23 ± 0.06 mg/dl	0.27 ± 0.25 mg/dl	-1.19	In-significant

***** $p < 0.001$**

Similarly, the mean concentration values for enzymes; alanine aminotransferase (ALT); 28.81 ± 5.63 IU/L and alkaline phosphatase (ALP); 211.25 ± 42.31 IU/L in HIV subjects of 18-29 years age group were significantly higher as compared to ALT (17.65 ± 6.55 IU/L) and ALP (147.59 ± 23.37 IU/L) respectively in normal subjects (control group). The *t*-test for enzymes; ALT and ALP show significant *t*-values of -8.27 ($p < 0.001$) and -8.82 ($p < 0.001$) respectively between the normal (control) and HIV subjects in 18-29 years age group.

The mean concentration of serum protein albumin (ALB) for HIV subjects (2.73 ± 0.39 g/dl) was lower as compared to the normal subjects (3.51 ± 0.52 g/dl) in the 18-29 years age group. The *t*-test for ALB shows highly significant *t*-value of 7.67 ($p < 0.001$) between normal and HIV subjects within the same age group. The mean concentration values for LFTs; Bilirubin total (Bil-T), 0.49 ± 0.36 mg/dl and Bilirubin direct (Bil-D); 0.27 ± 0.25 mg/dl in HIV subjects (Group $H_{18\text{-}29}$), were slightly higher as compared to the Bil-T (0.41 ± 0.15 mg/dl) and Bil-D (0.23 ± 0.06 mg/dl) respectively in normal subjects (control group) in the 18-29 years age group. The *t*-

test for Bil-T and Bil-D did not show significant differences between normal and HIV subjects as seen by *t*-values of -1.47 and -1.19 respectively within the same age group.

5.1.1.6 Liver Function Analysis in 30-39 years age group

The mean concentration and *t*-test analysis of LFTs in Normal and HIV subjects of 30-39 years age group is shown in table 5.7. The mean concentration of enzyme AST for HIV subjects (34.29 ± 6.70 IU/L) was higher as compared to the normal subjects (26.56 ± 10.38 IU/L) in 30-39 years age group. The *t*-test for AST was significant with *t*-value of -4.32 ($p < 0.001$) between the normal (control) and HIV subjects of the same age group.

Table 5.7 Mean concentration and *t*-test analysis of Liver Function in Normal and HIV subjects [Age Group: 30-39 years]

Liver Function Tests [Age Group: 30-39 years]					
S.No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		Normal (n=45)	**HIV** (n=55)		
1.	Aspartate aminotransferase	26.56 ± 10.38 IU/L	34.29 ± 6.70 IU/L	-4.32	Significant***
2.	Alanine aminotransferase	21.58 ± 10.38 IU/L	29.64 ± 5.56 IU/L	-4.69	Significant***
3.	Alkaline phosphatase	140.36 ± 26.56 IU/L	173.69 ± 32.93 IU/L	-5.60	Significant***
4.	Serum albumin	3.89 ± 0.50 g/dl	2.85 ± 0.38 g/dl	11.55	Significant***
5.	Bilirubin total	0.48 ± 0.12 mg/dl	0.54 ± 0.23 mg/dl	-1.63	In-significant
6.	Bilirubin direct	0.24 ± 0.05 mg/dl	0.28 ± 0.16 mg/dl	-1.70	In-significant

***** p < 0.001**

Similarly, the mean concentration values for enzymes; ALT (29.64 ± 5.56 IU/L) and ALP (173.69 ± 32.93 IU/L) in HIV subjects (Group $H_{30\text{-}39}$), were significantly higher as compared to the concentration of ALT (21.58 ± 10.38 IU/L) and ALP (140.36 ± 26.56 IU/L) respectively in normal (control) subjects of 30-39 years age. The *t*-test for enzymes; ALT and ALP shows significant *t*-values of -4.69

($p < 0.001$) and -5.60 ($p < 0.001$) respectively between the normal and HIV subjects of the same age group.

The mean concentration of ALB in HIV subjects (2.85 ± 0.38 g/dl) was also significantly lower as compared to the normal subjects (3.89 ± 0.50 g/dl) in the 30-39 years age group. The *t*-test for ALB was highly significant with *t*-value of 11.55 ($p < 0.001$) between normal (control) and HIV subjects within the same age group.

The mean concentration values of Bil-T (0.54 ± 0.23 mg/dl) and Bil-D (0.28 ± 0.16 mg/dl) in HIV subjects (Group H_{30-39}) of 30-39 years age were slightly higher as compared to Bil-T (0.48 ± 0.12 mg/dl) and Bil-D (0.24 ± 0.05 mg/dl) respectively in normal subjects. This increase in concentration of Bil-T and Bil-D was not significant as revealed by *t*-values of -1.63 and -1.70 respectively between normal (control) and HIV subjects.

5.1.1.7 Liver Function Analysis in 40-49 years age group

The mean concentration and *t*-test analysis of LFTs in normal and HIV subjects of 40-49 years age group are shown in table 5.8. The mean concentration values for enzymes AST (33.98 ± 7.20 IU/L) and ALT (29.47 ± 4.75 IU/L) in HIV subjects (Group H_{40-49}) in the 40-49 years age group were significantly higher as compared to the concentration of AST (22.78 ± 6.66 IU/L) and ALT (19.06 ± 6.22 IU/L) respectively in normal (control) subjects. The *t*-test for enzymes; AST and ALT shows significant *t*-values of -7.25 ($p < 0.001$) and -8.30 ($p < 0.001$) respectively between normal and HIV subjects within the same age group.

The mean concentration of enzyme ALP in HIV subjects (194.09 ± 73.92 IU/L) of 40-49 years age was higher as compared to the normal (control) subjects (141.78 ± 40.30 IU/L). The increase in concentration of ALP was significant with *t*-value of -4.05 ($p < 0.001$) between normal (control) and HIV groups.

The mean concentration of ALB in HIV subjects (2.82 ± 0.38 g/dl) was significantly lower as compared to the normal (control) subjects (3.42 ± 0.56 g/dl) in the 40-49 years age group. The increase in mean concentration of ALB was significant with *t*-value of 5.50 ($p < 0.001$) between normal (control) and HIV subjects of same age group.

The mean concentration values of LFTs; Bil-T (0.63 ± 0.35 mg/dl) and Bil-D (0.32 ± 0.23 mg/dl) in HIV subjects (Group H_{40-49}), of 40-49 years age were significantly higher as compared to the Bil-T (0.48 ± 0.13 mg/dl) and Bil-D (0.24 ± 0.05 mg/dl) respectively in normal (control) subjects. The *t*-test of Bil-T and Bil-D shows significant *t*-values of -2.67 ($p < 0.05$) and -2.44 ($p < 0.05$) respectively between normal and HIV subjects.

Table 5.8 Mean concentration and *t*-test analysis of Liver Function in Normal and HIV subjects [Age Group: 40-49 years]

Liver Function Tests [Age Group: 40-49 years]					
S.No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		Normal (n=36)	**HIV** (n=45)		
1.	Aspartate aminotransferase	22.78 ± 6.66 IU/L	33.98 ± 7.20 IU/L	-7.25	Significant***
2.	Alanine aminotransferase	19.06 ± 6.22 IU/L	29.47 ± 4.75 IU/L	-8.30	Significant***
3.	Alkaline phosphatase	141.78 ± 40.30 IU/L	194.09 ± 73.92 IU/L	-4.05	Significant***
4.	Serum albumin	3.42 ± 0.56 g/dl	2.82 ± 0.38 g/dl	5.50	Significant***
5.	Bilirubin total	0.48 ± 0.13 mg/dl	0.63 ± 0.35 mg/dl	-2.67	Significant*
6.	Bilirubin direct	0.24 ± 0.05 mg/dl	0.32 ± 0.23 mg/dl	-2.44	Significant*

*** p < 0.05, *** p < 0.001**

The results of Bil-T and Bil-D are significant as compared to results from the previous (lower age) groups. These results could be because of higher age of the subjects, which could result in higher formation of the bilirubin pigment at age greater than 40 years. Nevertheless the values of Bil-T and Bil-D in all the age groups of normal and HIV subjects shows results within the normal range of each pigment. For example, the normal range of Bil-T is 0.2-1.2 mg/dl and of Bil D is 0.1-0.4 mg/dl.

It is evident from the results of LFT comparison between normal (control) and HIV subjects of different age groups that the concentration of the enzymes, viz. aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were

significantly higher in HIV subjects compared to normal subjects. This reveals that the increased activity of these three liver enzymes is HIV induced. Serum protein albumin show significantly lower levels in HIV subjects as compared to normal subjects in all the three age groups. These results are more important because the values of different parameters in HIV subjects are significantly different than the normal values for the same parameters accepted at global level. For example, the normal ranges of LFTs are: 10-35 IU/L (AST), 9-40 IU/L (ALT), 30-120 IU/L (ALP) and 3.5-5.3 g/dl (ALB).

5.1.1.8 Liver Function Comparison between HIV and AIDS Subjects in 18-49 years age

Table 5.9 shows the mean concentration and *t*-test analysis of LFTs in HIV and AIDS subjects of 18-49 years age group from both the states.

Table 5.9 Mean concentration and *t*-test analysis of Liver Function in HIV and AIDS subjects [Age Group: 18-49 years]

Liver Function Tests [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD**		***t*-value**	**Significant/ Insignificant**
		HIV (n=148)	**AIDS** (n=53)		
1.	Aspartate aminotransferase	34.06 ± 6.70 IU/L	86.57 ± 65.97 IU/L	-5.78	Significant***
2.	Alanine aminotransferase	29.32 ± 5.33 IU/L	77.55 ± 76.14 IU/L	-4.61	Significant***
3.	Alkaline phosphatase	192.07 ± 53.43 IU/L	336.60 ± 148.06 IU/L	-6.95	Significant***
4.	Serum albumin	2.80 ± 0.38 g/dl	2.39 ± 0.35 g/dl	7.13	Significant***
5.	Bilirubin total	0.55 ± 0.32 mg/dl	0.52 ± 0.40 mg/dl	0.18	In-significant
6.	Bilirubin direct	0.29 ± 0.22 mg/dl	0.28 ± 0.26 mg/dl	0.23	In-significant

***** p < 0.001**

The mean concentration values of enzymes; AST (86.57 ± 65.97 IU/L), ALT (77.55 ± 76.14 IU/L) and ALP (336.60 ± 148.06 IU/L) in AIDS subjects (Group A_{18-49}), were significantly higher as compared to the AST (34.06 ± 6.70 IU/L), ALT (29.32 ± 5.33 IU/L) and ALP (192.07 ± 53.43 IU/L) in HIV subjects (Group H_{18-49}) of 18-49 years age. The *t*-test for enzymes; AST, ALT and ALP show significant *t*-values of -5.78 ($p < 0.001$), -4.61 ($p < 0.001$) and -6.95 ($p < 0.001$) respectively between HIV and AIDS subjects in the same age group. These results reveal that the activity of liver enzymes increases significantly with the progression of HIV infection into AIDS expression.

The graphical representation of LFT enzymes; AST, ALT and ALP is shown in figures 5.1, 5.2 and 5.3 respectively. The mean concentration values of normal, HIV and AIDS subjects under different groups for all the parameters under study are shown in the form of graphical representation. Each figure shows the corresponding changes in the concentration values of respective parameter in the form of a bar graph.

The mean concentration of AST shows increasing trends in all the age groups of HIV subjects (H_{18-29}, H_{30-39} and H_{40-49}) as compared to all the age groups of normal subjects (N_{18-29}, N_{30-39} and N_{40-49}). Similar trend of increase in mean concentration of AST was also observed in comparison of AIDS (A_{18-49}) and HIV ((H_{18-49}) subjects. Likewise, results were shown for enzymes ALT and ALP. The mean concentration values of ALT and ALP shows increasing trends in all the three age groups of HIV subjects (H_{18-29}, H_{30-39} and H_{40-49}) as compared to normal subjects (N_{18-29}, N_{30-39} and N_{40-49}). Similar trend of increase in mean concentration values for ALT and ALP was also observed in AIDS subjects (A_{18-49}) as compared to HIV subjects ((H_{18-49}).

These results show that there was a significant increase in the activity of liver enzymes; AST, ALT and ALP with the progress of HIV infection to AIDS expression. This indicates that the increased activity of liver enzymes was induced by HIV.

As shown in table 5.9, the mean concentration of ALB in AIDS subjects (2.39 ± 0.35 g/dl) was significantly lower as compared to the HIV subjects (2.80 ± 0.38

g/dl) in the 18-49 years age group. The *t*-test for ALB shows a highly significant *t*-value of 7.13 ($p < 0.001$) between HIV and AIDS subjects in the same age group. The Bil-T and Bil-D levels show in-significant results in terms of differences in mean concentration and *t*-value in the 18-49 years age group for HIV (Group H_{18-49}) and AIDS (Group A_{18-49}) subjects.

The graphical representation of serum protein albumin (ALB) is shown in figure 5.4. The mean concentration of ALB shows decreasing trends in all the age groups of HIV subjects (H_{18-29}, H_{30-39} and H_{40-49}) as compared to all the age groups of normal subjects (N_{18-29}, N_{30-39} and N_{40-49}). Similar trend of decreasing mean concentration for ALB was also observed in AIDS subjects (A_{18-49}) as compared to HIV subjects ((H_{18-49}).

It can be seen from the above table 5.9 and figures 5.1 to 5.4, that the increase in the activity of liver enzymes with a decrease in serum albumin is a function of HIV.

The graphical representation of LFTs Bil-T and Bil-D is shown in figures 5.5 and 5.6 respectively. The mean concentration values of Bil-T and Bil-D shows in-significant increasing trends in the lower age groups of HIV subjects (H_{18-29} and H_{30-39}) as compared to the lower age groups of normal subjects (N_{18-29} and N_{30-39}). Whereas the higher age group of HIV subjects (H_{40-49}) show significant increase in mean concentration values of Bil-T and Bil-D as compared to the normal subjects (N_{40-49}). This trend of increasing mean concentration was found to be reversed in AIDS subjects (A_{18-49}) where mean concentration values for Bil-T and Bil-D decreases (in-significantly) as compared to HIV subjects ((H_{18-49}).

The results of Bil-T and Bil-D indicate that their concentration values are affected marginally in an in-significant manner in AIDS and HIV subjects as compared to normal subjects.

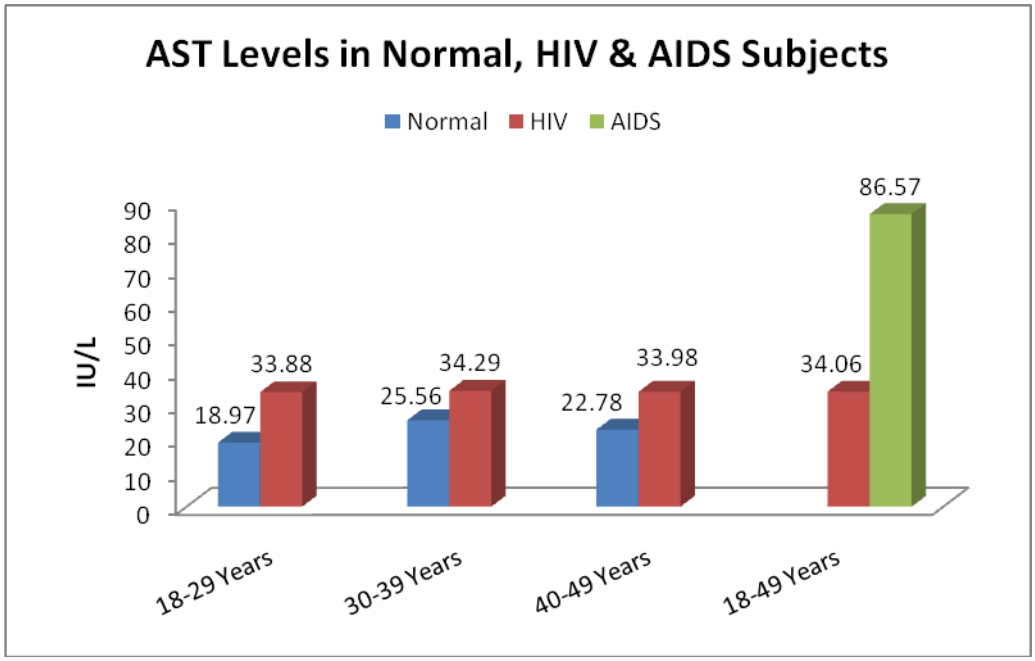

Figure 5.1 Mean concentration of Aspartate aminotransferase for normal, HIV and AIDS subjects

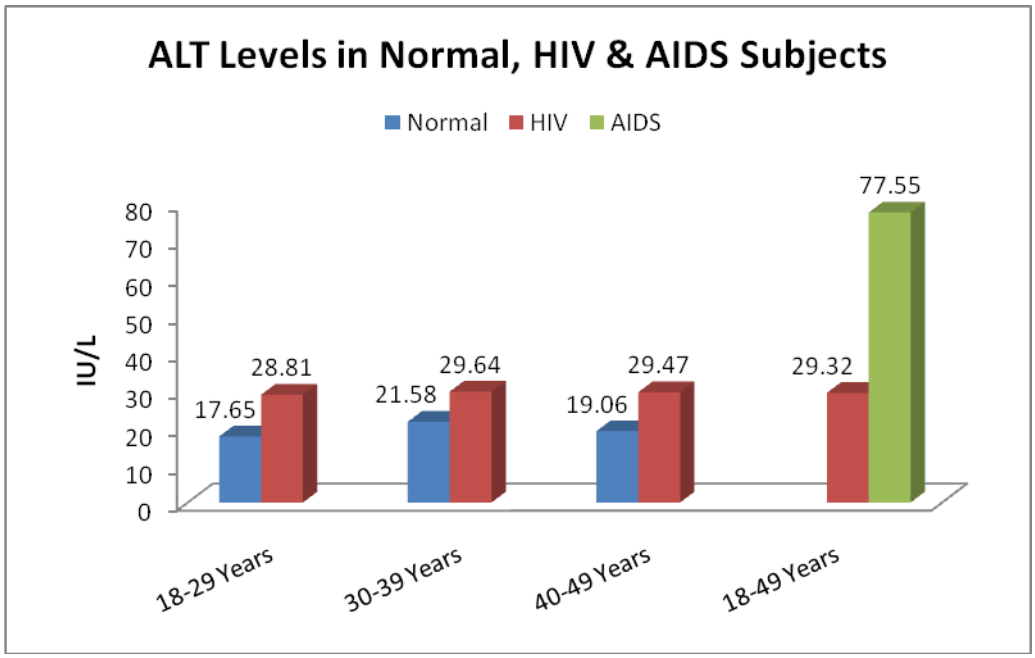

Figure 5.2 Mean concentration of Alanine aminotransferase for normal, HIV and AIDS subjects

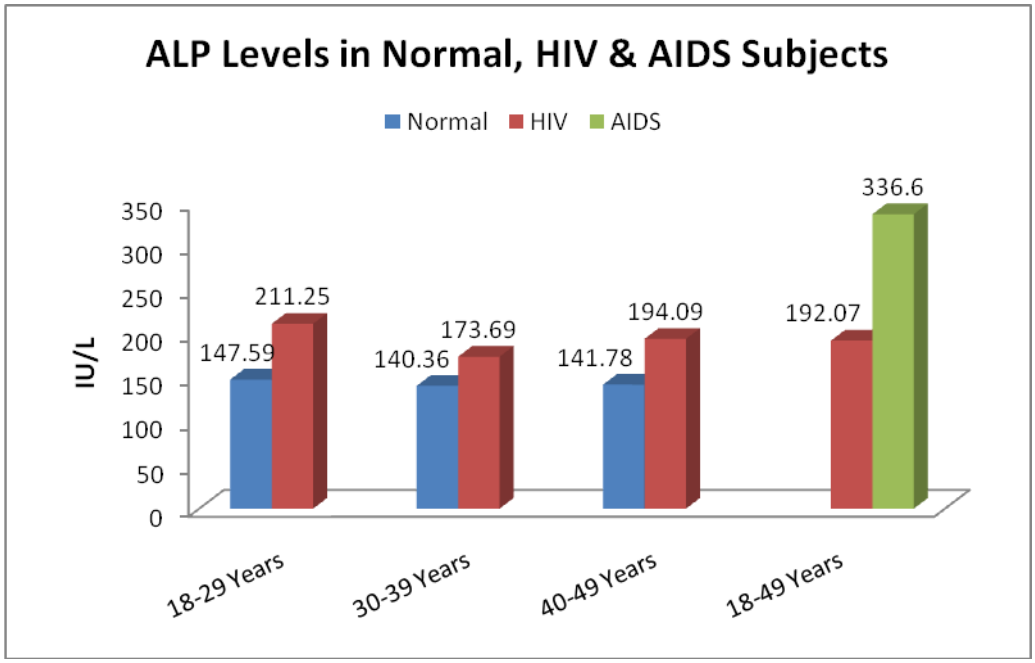

Figure 5.3 Mean concentration of Alkaline phosphatase for normal, HIV and AIDS subjects

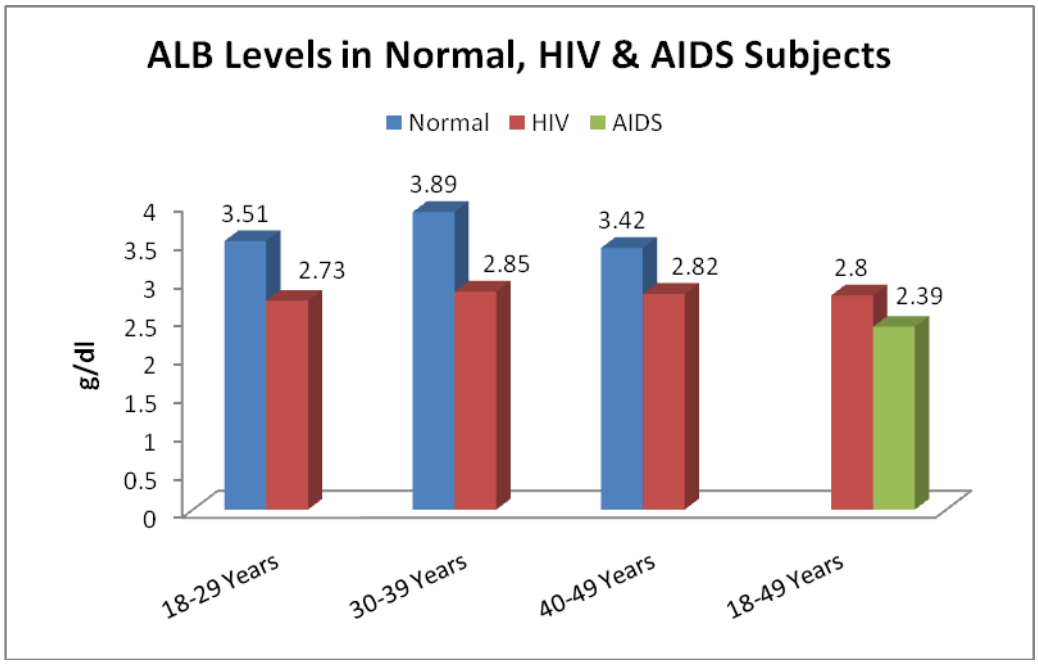

Figure 5.4 Mean concentration of Serum albumin for normal, HIV and AIDS subjects

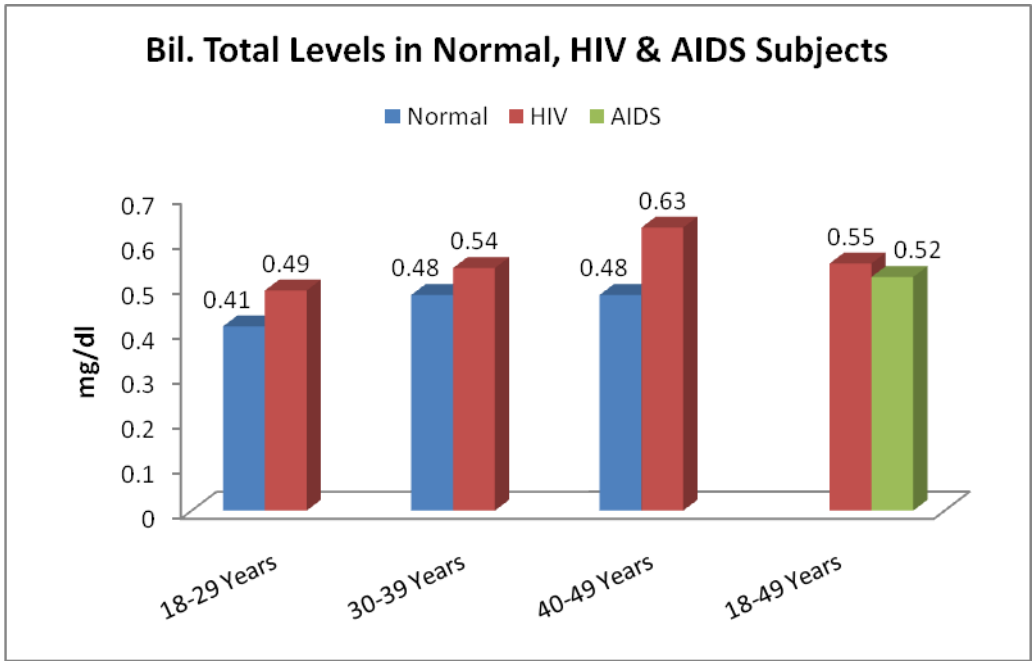

Figure 5.5 Mean concentration of Bilirubin Total for normal, HIV and AIDS subjects

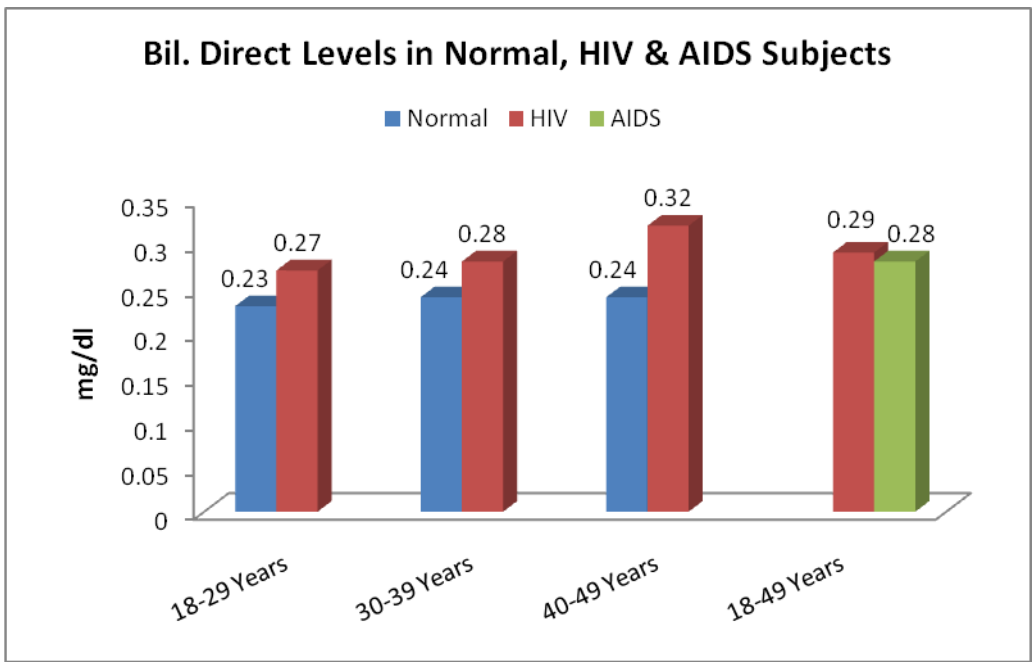

Figure 5.6 Mean concentration of Bilirubin Direct for normal, HIV and AIDS subjects

HIV infection leads to deviation and/or dysfunction of main metabolic functions in the body including liver function, kidney function and lipid profile. Therefore the analysis of LFTs, KFTs and lipid profile forms the clinical diagnostic parameter for HIV/AIDS [93, 101, 111, 124, 127].

The results of liver function analysis shown through above tables and figures, reveals that the activities of three liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increases in the HIV affected subjects as compared to the normal subjects. The increase in activity is significant in HIV +ve to AIDS subjects. Our results are consistent with report of Palanisamy et al. [101] on Tamilian HIV patients. Similar reports have been made for other populations from Nigeria [96], South Africa [201], Uganda [202], Mexico [92] and other world population [203, 204].

HIV attack host cells and take over the control of the infected cells leading to eventual death of the cells and subsequently the release of cellular contents into the surrounding medium of which enzymes constitute 20%. This may be responsible for the increase in the level of liver enzymes in infected liver cells. Changes in the AST, ALT and ALP enzymes could be due to effective immune response against viral replications which is necessary to produce the hepatocellular necrosis and inflammation seen in active hepatitis due to HIV infection and AIDS [101].

The elevated liver enzyme activities may also be attributed to HIV induced inflammatory responses [205] and /or due to HIV induced cell and tissue necrosis releasing intracellular enzymes in the blood. Possible mechanisms could be apoptosis (induced by caspases 2, 7 and 8) and mitochondrial dysfunction with decrease in mitochondrial DNA in several tissues [92].

Other causes of increased serum enzymes include: hepatitis due to hepatitis B virus, HCV, drug toxicity, extra-hepatic cholestasis, cirrhosis, hepatobiliary disease, genetic abnormalities with increased production of enzymes, enzyme induction and proliferation of enzyme producing cells, for example, in cancer patients. It may be possible, that these other conditions could be secondary to HIV infection and thus

contribute to an increase in the activities of the liver enzymes examined perhaps to different degrees of affect [106].

Further, our investigation shows that the increase in enzyme activities in HIV subjects is independent of topographic location of the subject or age groups.

Our results on decreased serum albumin concentration in HIV +ve subjects is in agreement with earlier reports on HIV affected subjects [96, 201, 206]. The conclusion that serum albumin decrease is significantly correlated to HIV infection is more or less parallel to that of Oosthuizen et al. [201] showing a significance level of $p < 0.01$. The decrease in albumin induced by HIV is independent of age and topographic location of the affected person.

Earlier, Emejulu et al. [96] reported an increase in bilirubin (Bil-T and Bil-D) concentration in HIV subjects compared to non-HIV subjects with a significance level of $p < 0.05$ in Nigerian population. Our results on bilirubin do not agree with the above report, but finds similarity with reports of Ebuehi et al. [95] on Nigerian population to the fact that there is no significant variation in bilirubin levels in HIV subjects compared to normal subjects.

5.1.2 Kidney Function Analysis

5.1.2.1 State – 1 (Punjab) Kidney Function Analysis in 18-49 years age group

The mean concentration and the *t*-test analysis of kidney function tests (KFTs) in normal and HIV subjects of 18-49 years in state-1 (Punjab) are shown in table 5.10. The mean concentration of urea (23.96 ± 9.56 mg/dl) in HIV subjects was significantly lower as compared to the normal subjects (27.79 ± 4.63 mg/dl) in state-1. The *t*-test for blood urea shows significant *t*-value of 2.60 ($p < 0.05$) between normal and HIV subjects within the same state. The mean concentration of creatinine (1.01 ± 0.32 mg/dl) in HIV subjects was lower as compared to the normal subjects (1.10 ± 0.17mg/dl) within the same state while the *t*-test for the same shows slightly in-significant with *t*-value of 1.98 between normal and HIV subjects.

Table 5.10 Mean concentration and *t*-test analysis of Kidney Function in Normal and HIV subjects from State-1 (Punjab)

Kidney Function Tests [State-1 (Punjab)] [Age Group: 18-49 years]					
S. No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=46)		
1.	Blood Urea	27.79 ± 4.63	23.96 ± 9.56	2.60	Significant*
2.	Serum Creatinine	1.10 ± 0.17	1.01 ± 0.32	1.98	In-significant

*** $p < 0.05$**

5.1.2.2 State – 2 (Rajasthan) Kidney Function Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of kidney function tests (KFTs) in normal and HIV subjects of 18-49 years in the state-2 (Rajasthan) are shown in table 5.11. The mean concentration values of urea and serum creatinine in HIV subjects shows very little difference as compared to the mean concentration values of normal subjects in state-2. The *t*-test for the same shows in-significant *t*-values of -0.27 and 0.99 respectively between normal and HIV subjects within the same state.

Table 5.11 Mean concentration and *t*-test analysis of Kidney Function in Normal and HIV subjects from State-2 (Rajasthan)

Kidney Function Tests [State-2 (Rajasthan)] [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=102)		
1.	Blood Urea	27.79 ± 4.63	28.22 ± 15.18	-0.27	In-significant
2.	Serum Creatinine	1.10 ± 0.17	1.07 ± 0.35	0.99	In-significant

5.1.2.3 Kidney Function Comparison between HIV Groups in State-1 and State-2

The comparison of the *t*-values of normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan) for analysis of KFTs is shown in table 5.12. The *t*-test results (for comparison between normal and HIV subjects) for both parameters in KFTs (blood urea and serum creatinine) shows in-significant and lower *t*-values in state-2. In comparison, state-1 shows higher *t*-values for *t*-tests with blood urea showing significant while serum creatinine showing marginally in-significant *t*-value.

Table 5.12 Comparison of *t*-values of Kidney Function Tests for Normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan)

Kidney Function Tests			
S. No.	**Tests**	***t*- value**	
		State-1	**State-2**
1.	Blood Urea	**2.60 (Significant)**	-0.27 (In-significant)
2.	Serum Creatinine	1.98 (In-significant)	0.99 (In-significant)

The mean concentration and *t*-test analysis of KFTs in HIV subjects of state-1 (Punjab) versus state-2 (Rajasthan) is shown in table 5.13. The mean concentration of blood urea (23.96 ± 9.56 mg/dl) in HIV subjects of state-1 was significantly lower as compared to the HIV subjects (28.22 ± 15.18 mg/dl) of state-2. The *t*-test for blood

urea shows significant *t*-value of -2.07 ($p < 0.05$) while the *t*-test for serum creatinine shows in-significant *t*-value of -1.04 between HIV subjects of state-1 and state-2.

Table 5.13 Mean concentration and *t*-test analysis of Kidney Function in HIV subjects of State-1 (Punjab) versus State-2 (Rajasthan)

Kidney Function Tests [State-1 versus State-2]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=46)	**HIV** (n=102)		
1.	Blood Urea	23.96 ± 9.56	28.22 ± 15.18	-2.07	Significant*
2.	Serum Creatinine	1.01 ± 0.32	1.07 ± 0.35	-1.04	In-significant

*** p < 0.05**

5.1.2.4 Age Group Analysis of Kidney Function

To assess the overall age based kidney function analysis, the data from the two states was combined and the total samples were divided into different age groups of normal, HIV and AIDS as shown earlier in table 5.5.

5.1.2.5 Kidney Function Analysis in 18-29 years age group

The mean concentration and *t*-test analysis of KFTs in normal and HIV subjects of 18-29 years age group is shown in table 5.14. The mean concentration values of blood urea (24.96 ± 8.85 mg/dl) and serum creatinine (1.07 ± 0.29 mg/dl) in HIV subjects (Group $H_{18\text{-}29}$) of 18-29 years age shows very little difference as compared to the mean concentration values of urea (24.86 ± 3.87 mg/dl) and serum creatinine (1.01 ± 0.13 mg/dl) respectively in normal subjects (Control Group $N_{18\text{-}29}$). The *t*-test for blood urea and serum creatinine shows in-significant *t*-values of -0.07 and -1.20 respectively between normal (control) and HIV subjects with in the same age group.

Table 5.14 Mean concentration and *t*-test analysis of Kidney Function in Normal and HIV subjects [Age Group: 18-29 years]

Kidney Function Tests [Age Group: 18-29 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=37)	**HIV** (n=48)		
1.	Blood Urea	24.86 ± 3.87	24.96 ± 8.85	-0.07	In-significant
2.	Serum Creatinine	1.01 ± 0.13	1.07 ± 0.29	-1.20	In-significant

5.1.2.6 Kidney Function Analysis in 30-39 years age group

The mean concentration and *t*-test analysis of KFTs in normal and HIV subjects of 30-39 years age group is shown in table 5.15. The mean concentration values of urea and serum creatinine in HIV subjects (Group $H_{30\text{-}39}$) of 30-39 years age shows very little difference as compared to the concentration values of normal subjects (Control Group $N_{30\text{-}39}$). The *t*-test for blood urea and serum creatinine shows in-significant *t*-values of -0.37 and -1.74 respectively (similar to the *t*-test results shown in 18-29 years age group) between normal (control) and HIV subjects within 30-39 years age group.

Table 5.15 Mean concentration and *t*-test analysis of Kidney Function in Normal and HIV subjects [Age Group: 30-39 years]

Kidney Function Tests [Age Group: 30-39 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=45)	**HIV** (n=55)		
1.	Blood Urea	28.73 ± 4.22	29.65 ± 17.70	-0.37	In-significant
2.	Serum Creatinine	1.13 ± 0.18	1.03 ± 0.39	1.74	In-significant

5.1.2.7 Kidney Function Analysis in 40-49 years age group

The mean concentration and *t*-test analysis of KFTs in 40-49 years age group (table 5.16) shows significant results as compared to mean concentration and *t*-test analysis of KFTs of lower age groups (table 5.14 and table 5.15) between normal and HIV subjects. The mean concentration values for urea (25.58 ± 12.34 mg/dl) and serum creatinine (1.05 ± 0.32 mg/dl) in HIV subjects (Group H_{40-49}) of age 40-49 years were significantly lower as compared to the mean concentration of urea (29.61 ± 4.50 mg/dl) and serum creatinine (1.17 ± 0.17 mg/dl) respectively in normal subjects (Control Group N_{40-49}). The *t*-test for blood urea and serum creatinine shows significant *t*-values of 2.03 ($p < 0.05$) and 2.07 ($p < 0.05$) respectively between normal (control) and HIV subjects within the same age group. These significant results may be attributed to the higher age of HIV subjects (40-49 years) in which kidney function deteriorates.

Table 5.16 Mean concentration and *t*-test analysis of Kidney Function in Normal and HIV subjects [Age Group: 40-49 years]

Kidney Function Tests [Age Group: 40-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=36)	**HIV** (n=45)		
1.	Blood Urea	29.61 ± 4.50	25.58 ± 12.34	2.03	Significant*
2.	Serum Creatinine	1.17 ± 0.17	1.05 ± 0.32	2.07	Significant*

*** $p < 0.05$**

5.1.2.8 Kidney Function Comparison between HIV and AIDS Subjects in 18-49 years age group

Table 5.17 shows the mean concentration and *t*-test analysis of KFTs in HIV and AIDS subjects of 18-49 years age group from both the states. The mean concentration values of blood urea and serum creatinine in HIV subjects (Group H_{18-49}) of age 18-49 years shows in-significant differences as compared to the values of normal subjects (Group N_{18-49}). The *t*-test for blood urea and serum creatinine shows

in-significant *t*-values of -0.19 and 0.66 respectively between HIV and AIDS subjects within the same age group.

Table 5.17 Mean concentration and *t*-test analysis of Kidney Function for comparison in HIV and AIDS subjects [Age Group: 18-49 years]

Kidney Function Tests [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=148)	**AIDS** (n=53)		
1.	Blood Urea	26.89 ± 13.79	27.30 ± 13.73	-0.19	In-significant
2.	Serum Creatinine	1.05 ± 0.34	1.02 ± 0.24	0.66	In-significant

The graphical representation of kidney function parameters; blood urea and serum creatinine is shown in figures 5.7 and 5.8 respectively. The mean concentration values of normal, HIV and AIDS subjects under different groups for all the parameters under study are shown in the form of graphical representation. Each figure shows the corresponding changes in the concentration values of respective parameter in the form of a bar graph.

The mean concentration of blood urea shows marginal increase in the lower age groups of HIV subjects ($H_{18\text{-}29}$ and $H_{30\text{-}39}$) as compared to the lower age groups of normal subjects ($N_{18\text{-}29}$ and $N_{30\text{-}39}$). The higher age group of HIV subjects ($H_{40\text{-}49}$) show significant decrease in the mean concentration of blood urea as compared to the higher age group of normal subjects ($N_{40\text{-}49}$). The reverse trend of marginal increase in mean concentration of blood urea was observed in AIDS subjects ($A_{18\text{-}49}$) as compared to HIV subjects (($H_{18\text{-}49}$).

The mean concentration of serum creatinine shows slight increase in the lower age group of HIV subjects ($H_{18\text{-}29}$) as compared to the normal subjects ($N_{18\text{-}29}$). The 30-39 years age group of HIV subjects ($H_{30\text{-}39}$) show in-significant decrease in the mean concentration of serum creatinine as compared to the normal subjects ($N_{30\text{-}39}$). Whereas the higher age group of HIV subjects ($H_{40\text{-}49}$) show significant decrease in

the mean concentration of serum creatinine as compared to the higher age group of normal subjects (N_{40-49}). The mean concentration of serum creatinine decrease marginally in an in-significant manner in AIDS subjects (A_{18-49}) as compared to HIV subjects ((H_{18-49}).

Our analysis of kidney function parameters; blood urea and creatinine in different HIV and normal groups show that the blood urea levels of HIV +ve and HIV –ve subjects did not differ significantly in subjects from Punjab and Rajasthan as well as in relation to age except that blood urea and creatinine levels of HIV +ve subjects differed significantly from the normal subjects. Comparison of blood urea and creatinine level between HIV +ve and AIDS expressed individuals also reveal in-significant differences.

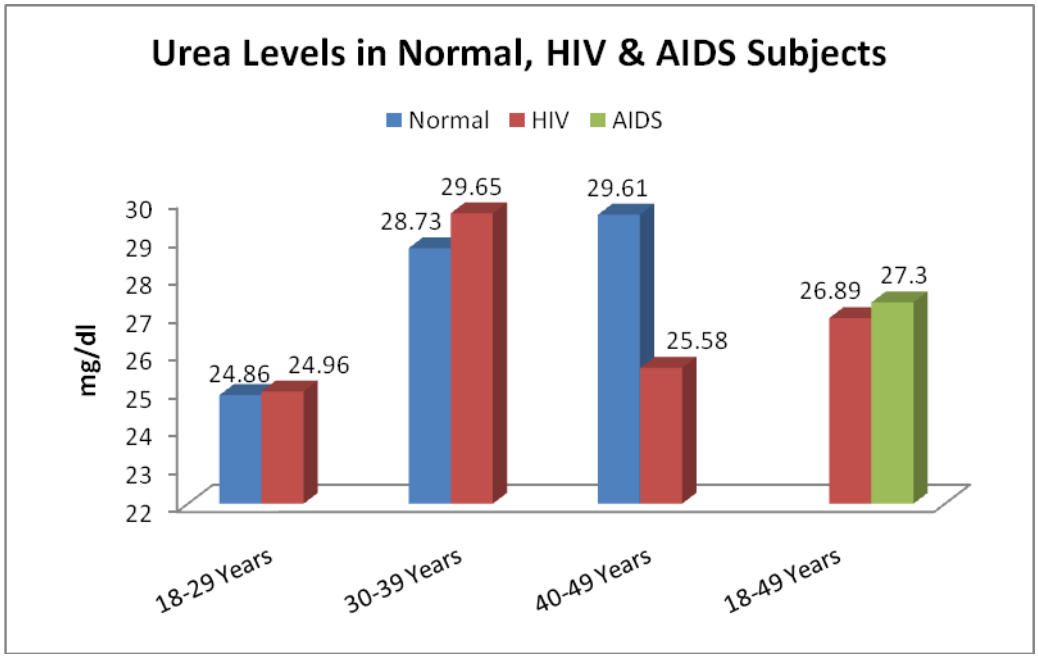

Figure 5.7 Mean concentration of Blood Urea for normal, HIV and AIDS subjects

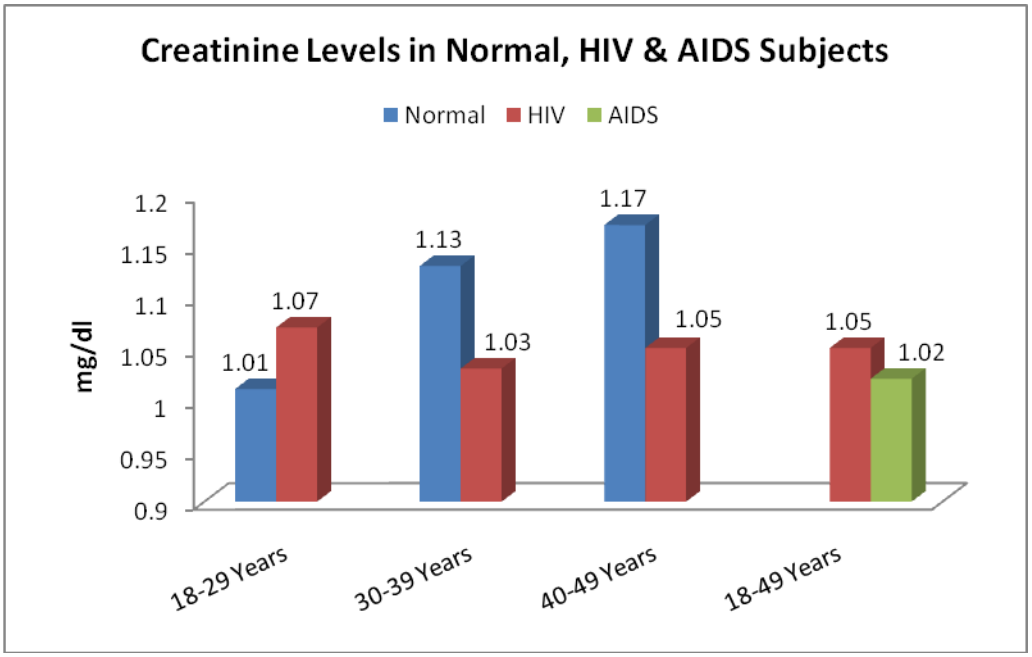

Figure 5.8 Mean concentration of Serum Creatinine for normal, HIV and AIDS subjects

Our results of kidney function are in agreement with the findings in South Indian population [121]. Earlier report by Afhami et al. [114] on blood urea and creatinine in Iranian population is parallel to our finding. In-significant difference in the serum creatinine level between HIV +ve and HIV –ve Nigerian subjects has also been reported recently [109]. However, significant increase in blood urea level of HIV +ve subjects has been reported in Nigerian and Cameroon population by few workers [207, 113].

Many workers have attributed anomalies in blood urea and creatinine level renal dysfunction with and without HIV infection [109, 114]. A higher creatinine level was independently associated with more-rapid progression to an incident AIDS-defining illness (ADI) among women after the initiation of HAART and, also, with an increased risk of death among women before the widespread use of HAART [116]. This assumes a clear association between proteinuria and the development of ADI among women before the widespread use of HAART.

5.1.3 Lipid Function Analysis

5.1.3.1 State – 1 (Punjab) Lipid Function Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of lipid function tests in normal and HIV subjects of 18-49 years age group from state-1 (Punjab) are shown in table 5.18. The mean concentration of serum cholesterol (TC); 138.07 ± 37.13 mg/dl in HIV subjects was lower as compared to the TC (150.10 ± 24.68 mg/dl) in normal subjects in state-1. The mean concentration of serum triglycerides (TG); 203.59 ± 63.79 mg/dl) in HIV subjects was significantly higher as compared to the TG (119.01 ± 16.58 mg/dl) in normal subjects within the same state. The *t*-test for TC and TG shows significant *t*-values of 2.03 ($p < 0.05$) and -8.88 ($p < 0.001$) respectively between normal and HIV subjects in state-1.

Table 5.18 Mean concentration and *t*-test analysis of Lipid Function in Normal and HIV subjects from State-1 (Punjab)

Lipid Function Tests [State-1 (Punjab)] [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=46)		
1.	Total Serum Cholesterol	150.10 ± 24.68	138.07 ± 37.13	2.03	Significant*
2.	Serum Triglycerides	119.01 ± 16.58	203.59 ± 63.79	-8.88	Significant***

*** p < 0.05, *** p < 0.001**

5.1.3.2 State -2 (Rajasthan) Lipid Function Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of lipid function tests in normal and HIV subjects of 18-49 years age group from state-2 (Rajasthan) are shown in Table 5.19. The mean concentration of TC (132.85 ± 35.85mg/dl) in HIV subjects was lower as compared to the TC (150.10 ± 24.68 mg/dl) in normal subjects in state-2. The mean concentration of TG (197.03 ± 51.97 mg/dl) in HIV subjects was significantly higher as compared to the TG (119.01 ± 16.58 mg/dl) in normal subjects within the same state. The *t*-test for TC and TG shows significant *t*-values of 4.09 (p <

0.001) and -14.54 ($p < 0.001$) respectively between normal and HIV subjects within the same state.

Table 5.19 Mean concentration and *t*-test analysis of Lipid Function in Normal and HIV subjects from State-2 (Rajasthan)

Lipid Function Tests [State-2 (Rajasthan)] [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=102)		
1.	Total Serum Cholesterol	150.10 ± 24.68	132.85 ± 35.85	4.09	Significant***
2.	Serum Triglycerides	119.01 ± 16.58	197.03 ± 51.97	-14.54	Significant***

***** p < 0.001**

5.1.3.3 Lipid Function Comparison between HIV Groups in State-1 and State-2

The comparison of the *t*-values of normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan) for analysis of lipid function tests is shown in table 5.20. The comparison of *t*-value between normal and HIV subjects for both lipid parameters (TC and TG) shows significant results in both state-1 and state-2.

Table 5.20 Comparison of *t*-values of Lipid Function for Normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan)

Lipid Function Tests			
S. No.	**Tests**	***t*- value**	
		State-1	**State-2**
1.	Total Serum Cholesterol	2.03 (Significant)	4.09 (Significant)
2.	Serum Triglycerides	-8.88 (Significant)	-14.54 (Significant)

The *t*-values for *t*-test in state -2 show higher ranges for both lipid parameters as compared to state-1.

The mean concentration and *t*-test analysis of lipid function tests in HIV subjects of state-1 (Punjab) versus state-2 (Rajasthan) is shown in table 5.21. The

differences in the mean concentration of TC and TG in HIV subjects between state-1 and state-2 are in-significant with *t*-values of 0.80 and 0.61 respectively.

Table 5.21 Mean concentration and *t*-test analysis of Lipid Function in HIV subjects of State-1 (Punjab) versus State-2 (Rajasthan)

Lipid Function Tests [State-1 versus State-2]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=46)	**HIV** (n=102)		
1.	Total Serum Cholesterol	138.07 ± 37.13	132.85 ± 35.85	0.80	In-significant
2.	Serum Triglycerides	203.59 ± 63.79	197.03 ± 51.97	0.61	In-significant

5.1.3.4 Age Group Analysis of Lipid Function

To assess the overall age based lipid function analysis, the data from the two states was combined and the total samples were divided into different age groups of normal, HIV and AIDS as shown earlier in table 5.5.

5.1.3.5 Lipid Function Analysis in 18-29 years age group

Table 5.22 shows the mean concentration and *t*-test analysis of LFTs in normal and HIV subjects of 18-29 years age group. The mean concentration of TC (137.31 ± 44.26 mg/dl) in HIV subjects ($H_{18\text{-}29}$ group) was slightly lower as compared to the TC (144.22 ± 19.55 mg/dl) in normal subjects (Control group $N_{18\text{-}29}$). The *t*-test for TC shows in-significant *t*-value of 0.97 between the same groups.

The mean concentration of TG (213.75 ± 69.28 g/dl) in HIV subjects was significantly higher as compared to the TG (113.84 ± 16.39 g/dl) in normal (control) subjects of 18-29 years age. The *t*-test for TG shows highly significant *t*-value of -9.65 ($p < 0.001$) between normal (control) and HIV subjects.

Table 5.22 Mean concentration and *t*-test analysis of Lipid Function in Normal and HIV subjects [Age Group: 18-29 years]

Lipid Function Tests [Age Group: 18-29 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=37)	**HIV** (n=48)		
1.	Total Serum Cholesterol	144.22 ± 19.55	137.31 ± 44.26	0.97	In-significant
2.	Serum Triglycerides	113.84 ± 16.39	213.75 ± 69.28	-9.65	Significant***

***** p < 0.001**

5.1.3.6 Lipid Function Analysis in 30-39 years age group

The mean concentration and *t*-test analysis of LFTs in normal and HIV subjects of 30-39 years age group is shown in table 5.23. The mean concentration of TC (134.11 ± 35.57 mg/dl) in HIV subjects (Group H_{30-39}), was significantly lower as compared to TC (152.33 ± 25.45 mg/dl) in the normal subjects (Control group N_{30-39}). The mean concentration of TG (191.98 ± 43.94 mg/dl) in HIV subjects (Group H_{30-39}) was significantly higher as compared to TG (118.18 ± 16.20 mg/dl) in normal subjects (Control group N_{30-39}). The *t*-test for TC and TG shows significant *t*-values of 2.98 ($p < 0.001$) and -11.54 ($p < 0.001$) respectively between normal (control) and HIV subjects in the 30-39 years age group.

Table 5.23 Mean concentration and *t*-test analysis of Lipid Function in Normal and HIV subjects [Age Group: 30-39 years]

Lipid Function Tests [Age Group: 30-39 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=45)	**HIV** (n=55)		
1.	Total Serum Cholesterol	152.33 ± 25.45	134.11 ± 35.57	2.98	Significant**
2.	Serum Triglycerides	118.18 ± 16.20	191.98 ± 43.94	-11.54	Significant***

**** p < 0.01, *** p < 0.001**

5.1.3.7 Lipid Function Analysis in 40-49 years age group

The mean concentration and *t*-test analysis of lipid function tests in Normal and HIV subjects of 40-49 years age group is shown in table 5.24.

The mean concentration of TC (131.89 ± 26.81 mg/dl) in HIV subjects (Group H_{40-49}), was significantly lower as compared to the TC (153.36 ± 27.83 mg/dl) in normal subjects (Control group N_{40-49}). The mean concentration of TG (192.07 ± 50.46 mg/dl) in HIV subjects (Group H_{40-49}), was significantly higher as compared to the TG (125.36 ± 15.58 mg/dl) in normal subjects (Control group N_{40-49}). The *t*-test for TC and TG shows significant *t*-values of 3.51 ($p < 0.001$) and -8.38 ($p < 0.001$) respectively between normal (control) and HIV subjects in the 40-49 years age group.

Table 5.24 Mean concentration and *t*-test analysis of Lipid Function in Normal and HIV subjects [Age Group: 40-49 years]

Lipid Function Tests [Age Group: 40-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=36)	**HIV** (n=45)		
1.	Total Serum Cholesterol	153.36 ± 27.83	131.89 ± 26.81	3.51	Significant***
2.	Serum Triglycerides	125.36 ± 15.58	192.07 ± 50.46	-8.38	Significant***

***** p < 0.001**

5.1.3.8 Lipid Function Comparison between HIV and AIDS Subjects in 18-49 years age group

Table 5.25 shows the mean concentration and *t*-test analysis of lipid function tests in HIV and AIDS subjects of 18-49 years age group from both the states.

The mean concentration of TC (114.66 ± 31.97 mg/dl) in AIDS subjects (Group A_{18-49}), was significantly lower as compared to the TC (134.47 ± 36.21 mg/dl) in HIV subjects (Group H_{18-49}). The mean concentration of TG (251.58 ± 66.39 mg/dl) in AIDS subjects (Group A_{18-49}), was significantly higher as compared to the TG (199.07 ± 55.77 mg/dl) in HIV subjects (Group H_{18-49}). The *t*-test for TC and TG shows

significant *t*-values of 3.74 ($p < 0.001$) and -5.15 ($p < 0.001$) respectively between HIV and AIDS subjects in the 18-49 years age group.

Table 5.25 Mean concentration and *t*-test analysis of Lipid Function in HIV and AIDS subjects [Age Group: 18-49 years]

Lipid Function Tests [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=148)	**AIDS** (n=53)		
1.	Total Serum Cholesterol	134.47 ± 36.21	114.66 ± 31.97	3.74	Significant***
2.	Serum Triglycerides	199.07 ± 55.77	251.58 ± 66.39	-5.15	Significant***

***** p < 0.001**

The graphical representation of lipid parameters; total serum cholesterol (TC) and serum triglycerides (TG) are shown in figures 5.9 and 5.10 respectively. The mean concentration values of normal, HIV and AIDS subjects under different groups for all the parameters under study are shown in the form of graphical representation. Each figure shows the corresponding changes in the concentration values of respective parameter in the form of a bar graph.

The mean concentration of TC shows decreasing trends in all the age groups of HIV subjects ($H_{18\text{-}29}$, $H_{30\text{-}39}$ and $H_{40\text{-}49}$) as compared to all the age groups of normal (control) subjects ($N_{18\text{-}29}$, $N_{30\text{-}39}$ and $N_{40\text{-}49}$). Similar trend of decreasing mean concentration for TC was also observed in AIDS subjects ($A_{18\text{-}49}$) as compared to HIV subjects (($H_{18\text{-}49}$).

The mean concentration of TG shows increasing trends with very high significance in all the age groups of HIV subjects ($H_{18\text{-}29}$, $H_{30\text{-}39}$ and $H_{40\text{-}49}$) as compared to all the age groups of normal (control) subjects ($N_{18\text{-}29}$, $N_{30\text{-}39}$ and $N_{40\text{-}49}$). Similar trend of significant increase in mean concentration for TG was also observed in AIDS subjects ($A_{18\text{-}49}$) as compared to HIV subjects (($H_{18\text{-}49}$).

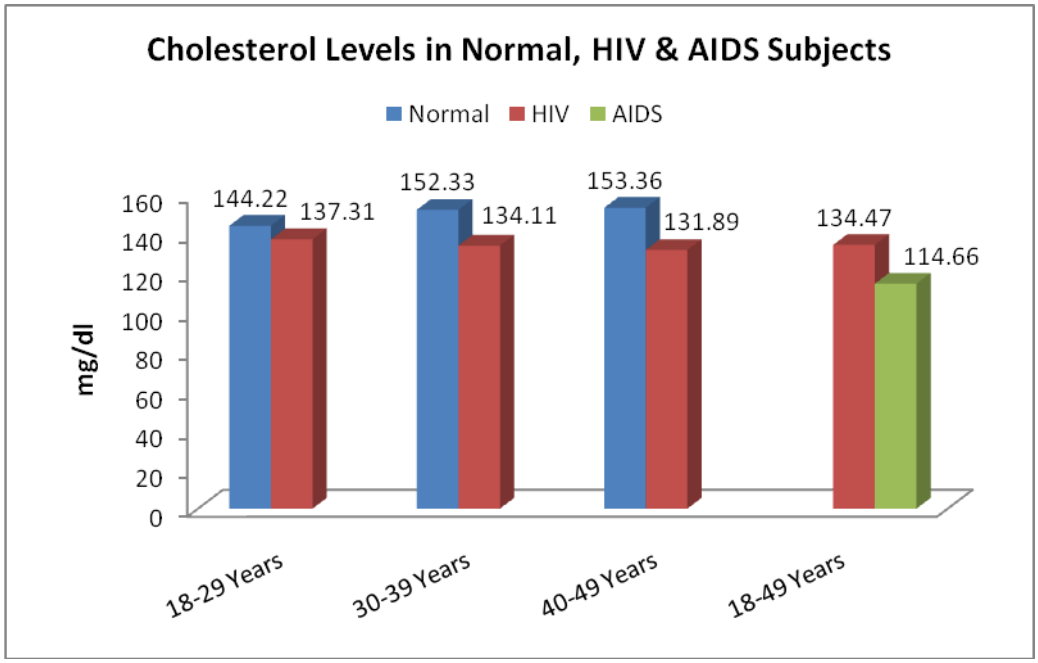

Figure 5.9 Mean concentration of Total serum cholesterol for normal, HIV and AIDS subjects

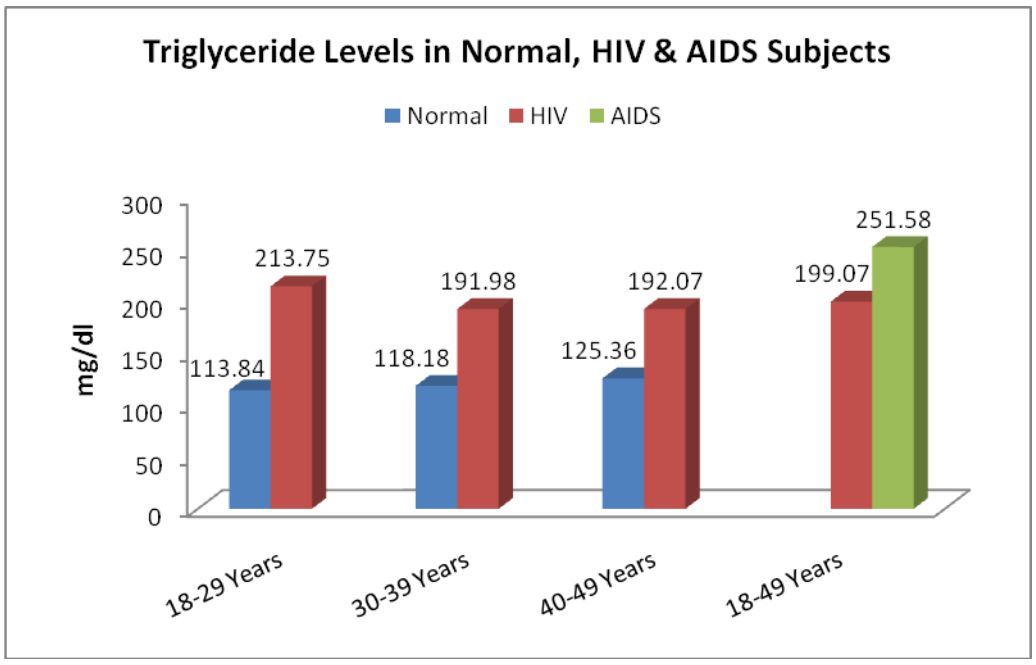

Figure 5.10 Mean concentration of Serum triglyceride for normal, HIV and AIDS subjects

The concentration analysis of total serum cholesterol (TC) and serum triglyceride (TG) in the present study, shows significant decrease of cholesterol with a significant increase in triglyceride in blood of HIV subjects compared to normal subjects irrespective of topographical locations and age. Further, results showing a significant decrease in TC and increase in TG suggest that these anomalies are HIV induced.

Earlier studies on the lipid profile of HIV +ve individuals have reported a variety of abnormalities in their lipid profiles. As in present investigation, most of the other investigators have reported a decrease in total cholesterol with an increase in triglyceride level in their study of population in different countries, viz; Nigeria [125, 130, 208, 209], Uganda [210], Switzerland [211], United State of America [212] and India [124, 127, 213]. Our observation of decrease in TC level and increase in TG level is consistent with the earlier reports. The increasing TG and decreasing TC behavior in HIV +ve subjects has been significantly correlated with CD-4^+ cell count by a host of workers [124, 125, 130, 214]. Earlier, Grunfeld [215, 216] reported, that the TC and TG abnormalities in HIV +ve subjects are also associated with increase in cytokine level. Gadd [217] reported racial varieties in serum lipid profile of HIV +ve individuals.

Alteration of plasma lipids have been reported since the first years of the HIV epidemic and are observed in both naïve patients and in those on treatment. Its development involves multiple factors, among which are those directly related to the ART, inflammation, hormonal and other not well defined genetic factors [123]. The HIV related dyslipidemia is characterized primarily by two factors: elevated plasma triglycerides (TG) and decreased HDL cholesterol (HDL). This pattern of low HDL and high TG is associated with small, dense LDL particles, and it has been also described as atherogenic dyslipidemia [133]. Lipid alterations may be the result of a reduction in the catabolism of very low density lipoprotein (VLDL). Other mechanisms potentially responsible for dyslipidemia are the reduction in the hydrolysis of triglyceride-rich lipoprotein [129], impaired catabolism of free fatty acids [131] and a reduction in free fatty acid trapping [132].

5.1.4 Blood Glucose Analysis

5.1.4.1 State – 1 (Punjab) Blood Glucose Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of random blood glucose test in normal and HIV subjects of 18-49 years age group from state-1 (Punjab) is shown in table 5.26. The mean concentration of blood glucose shows marginal difference between normal and HIV subjects with an in-significant *t*-value of 0.96 in state-1.

Table 5.26 Mean concentration and *t*-test analysis of Blood Glucose in Normal and HIV subjects from State-1 (Punjab)

Blood Glucose Test [State-1 (Punjab)] [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=46)		
1.	Blood Glucose (Random)	78.69 ± 18.99	76.09 ± 13.95	0.96	In-significant

5.1.4.2 State – 2 (Rajasthan) Blood Glucose Analysis in 18-49 years age group

The mean concentration and *t*-test analysis of random blood glucose test in normal and HIV subjects of 18-49 years age group from state-2 (Rajasthan) is shown in table 5.27. The mean concentration of blood glucose shows very in-significant difference between normal and HIV subjects with an in-significant *t*-value of 1.36 in state-2.

Table 5.27 Mean concentration and *t*-test analysis of Blood Glucose in Normal and HIV subjects from State-2 (Rajasthan)

Blood Glucose Test [State-2 (Rajasthan)] [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=118)	**HIV** (n=102)		
1.	Blood Glucose (Random)	78.69 ± 18.99	75.32 ± 17.69	1.36	In-significant

5.1.4.3 Blood Glucose Comparison between HIV Groups in State-1 and State-2

The comparison of the *t*-values of normal versus HIV subjects in state-1 (Punjab) and state-2 (Rajasthan) for analysis of blood glucose test is shown in table 5.28. The results of *t*-test (for analysis of random blood glucose concentration) show in-significant *t*-values in both state-1 and state-2. The *t*-value in state -2 shows higher value as compared to state-1.

Table 5.28 Comparison of *t*-values of Blood Glucose for Normal versus HIV subjects in State-1 (Punjab) and State-2 (Rajasthan)

Blood Glucose Test			
S. No.	**Tests**	***t*- value**	
		State-1	**State-2**
1.	Blood Glucose (Random)	0.96 (In-significant)	1.36 (In-significant)

The mean concentration and *t*-test analysis of blood glucose test in HIV subjects of state-1 (Punjab) versus state-2 (Rajasthan) is shown in table 5.29. The mean value of random blood glucose concentration shows marginal difference between HIV subjects of state-1 and state-2. The *t*-test for random blood glucose shows in-significant *t*-value of 0.28 between the groups of HIV subjects in state-1 and state-2.

Table 5.29 Mean concentration and *t*-test analysis of Blood Glucose in HIV subjects of State-1 (Punjab) versus State-2 (Rajasthan)

Blood Glucose Test [State-1 versus State-2]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=46)	**HIV** (n=102)		
1.	Blood Glucose (Random)	76.09 ± 13.95	75.32 ± 17.69	0.28	In-significant

5.1.4.4 Age Group Analysis of Blood Glucose

To assess the overall age based glucose function analysis, the data from the two states was combined and the total samples were divided into different age groups of normal, HIV and AIDS as shown earlier in table 5.5.

5.1.4.5 Blood Glucose Analysis in 18-29 years age group

Table 5.30 shows the mean concentration and *t*-test analysis of random blood glucose in normal and HIV subjects of 18-29 years age group. The mean concentration of blood glucose shows practically no difference with an in-significant *t*-value of 0.26 between normal (control) and HIV subjects in the 18-29 years age group.

Table 5.30 Mean concentration and *t*-test analysis of Blood Glucose in Normal and HIV subjects [Age Group: 18-29 years]

Blood Glucose Test [Age Group: 18-29 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=37)	**HIV** (n=48)		
1.	Blood Glucose (Random)	70.73 ± 8.72	70.23 ± 8.54	0.26	In-significant

5.1.4.6 Blood Glucose Analysis in 30-39 years age group

The mean concentration and *t*-test analysis of random blood glucose in normal and HIV subjects of 30-39 years age group is shown in table 5.31. The mean concentration of blood glucose shows very in-significant difference (similar to results in 18-29 years age group) with an in-significant *t*-value of 0.61 between normal (control) and HIV subjects in the 30-39 years age group.

Table 5.31 Mean concentration and *t*-test analysis of Blood Glucose in Normal and HIV subjects [Age Group: 30-39 years]

Blood Glucose Test [Age Group: 30-39 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=45)	**HIV** (n=55)		
1.	Blood Glucose (Random)	79.00 ± 17.66	76.96 ± 15.20	0.61	In-significant

5.1.4.7 Blood Glucose Analysis in 40-49 years age group

The mean concentration and *t*-test analysis of random blood glucose in normal and HIV subjects of 40-49 years age group is shown in table 5.32. The mean concentration of blood glucose shows very less difference (similar to results in lower age groups; 18-29 years and 30-39 years) with an in significant *t*-value of 1.31 between normal (control) and HIV subjects in the 40-49 years age group.

Table 5.32 Mean concentration and *t*-test analysis of Blood Glucose in Normal and HIV subjects [Age Group: 40-49 years]

Blood Glucose Test [Age Group: 40-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		Normal (n=36)	**HIV** (n=45)		
1.	Blood Glucose (Random)	86.47 ± 24.62	79.53 ± 22.52	1.31	In-significant

5.1.4.8 Blood Glucose Comparison between HIV and AIDS Subjects in 18-49 years age group

Table 5.33 shows the mean concentration and *t*-test analysis of blood glucose in HIV and AIDS subjects of 18-49 years age group. The mean concentration of blood

glucose shows very marginal difference with a highly in-significant *t*-value of -0.16 between HIV and AIDS subjects in the 18-49 years age group.

Table 5.33 Mean concentration and *t*-test analysis of Blood Glucose for comparison in HIV and AIDS subjects [Age Group: 18-49 years]

Blood Glucose Test [Age Group: 18-49 years]					
S.No.	**Tests**	**Mean ± SD (mg/dl)**		***t*-value**	**Significant/ In-significant**
		HIV (n=148)	**AIDS** (n=53)		
1.	Blood Glucose (Random)	75.56 ± 16.58	76.11 ± 23.39	-0.16	In-significant

The graphical representation of random blood glucose concentration is shown in figure 5.11. The mean concentration values of random blood glucose in normal, HIV and AIDS subjects under different age groups are shown in the form of graphical representation. Each figure shows the corresponding changes in the values of respective parameter in the form of a bar graph. The mean random blood glucose concentration shows marginal and in-significant decreasing trends in all the age groups of HIV subjects ($H_{18\text{-}29}$, $H_{30\text{-}39}$ and $H_{40\text{-}49}$) as compared to all the age groups of normal subjects ($N_{18\text{-}29}$, $N_{30\text{-}39}$ and $N_{40\text{-}49}$). Whereas the reverse trend of marginal and in-significant increase in mean random blood glucose concentration was observed in AIDS subjects ($A_{18\text{-}49}$) as compared to HIV subjects (($H_{18\text{-}49}$).

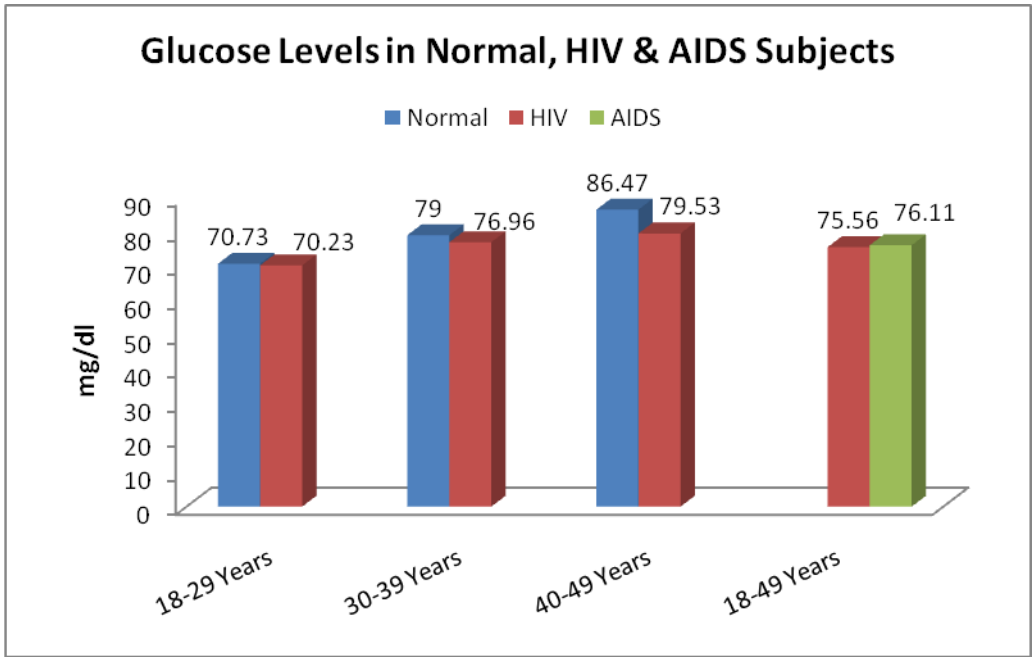

Figure 5.11 Mean concentration of Blood Glucose for normal, HIV and AIDS subjects

For the past 20 years, researchers have reported changes in glucose homeostasis in patients with HIV/AIDS [218]. Earlier reports stated that symptomatic HIV infected individuals had higher rates of insulin clearance and increased insulin sensitivity in peripheral tissues compared to non-infected individuals [219, 220]. The increase in non-insulin mediated glucose uptake in HIV infected subjects has been accounted for – by an increase in non-oxidative glucose disposal and is reported by Mulligan et al. [221]. According to them, the glucose production from liver tends to increase but the glucose cycle does not change in HIV +ve individuals. There is also evidence suggesting that insulin resistance may have HIV associated components. It is therefore reasonable to recommend general measures to increase insulin sensitivity in all patients infected with HIV, such as weight reduction for obese persons and regular aerobic exercise [222].

Dyslipidemia in patients with HIV infection is a marker of insulin resistance in liver, skeletal muscle, and adipose tissue. Moreover, decreased plasma adiponectin and increased plasma IL-6 concentrations were associated with impaired insulin-mediated suppression of endogenous glucose production and stimulation of glucose

disposal, suggesting that alterations in adipokine production might contribute to insulin resistance [131].

The observation in present investigation that the blood glucose level is more or less similar in HIV +ve and AIDS patients is in conformity with the earlier reports [218-224].

5.2 $CD4^+$ T Lymphocyte Analysis

5.2.1 $CD4^+$ T Lymphocyte ($CD4^+$) Count

Table 5.34 shows the characteristics of $CD4^+$ cell count in different age groups of HIV+ ve and AIDS subjects considered in the present investigation. The minimum and maximum $CD4^+$ cell count in HIV +ve subjects was 271 and 473 cells / µl respectively, with an average of 369.78 ± 41.48 cells / µl. The minimum and maximum $CD4^+$ cell count in AIDS subjects was 76 and 197 cells / µl respectively, with an average of 145.42 ± 29.55 cells / µl.

Table 5.34 $CD4^+$ Cell Count Characteristics of HIV +ve and AIDS Subjects

S. No.	Name of Groups	Age Groups	$CD4^+$ Cell Count / µl		
			Minimum	Maximum	Average (Mean ± SD)
1.	HIV (H_{18-29})	18-29 years	271	473	365.60 ± 45.46
2.	HIV (H_{30-39})	30-39 years	286	450	376.69 ± 35.66
3.	HIV (H_{40-49})	40-49 years	297	469	365.78 ± 43.42
4.	HIV (H_{18-49})	18-49 years	271	473	369.78 ± 41.48
5.	AIDS (A_{18-49})	18-49 years	76	197	145.42 ± 29.55

5.2.2 $CD4^+$ T Lymphocyte Correlation with Biochemical Parameters

The correlation of different parameters of study [(liver function tests (LFTs), kidney function tests (KFT's), lipid function tests and blood glucose)] with $CD4^+$ cell count is shown in table 5.35. The analysis includes HIV subjects from both the states [Punjab (State – 1) and Rajasthan (State – 2)]

Table 5.35 Correlation between Different Biochemical Parameters and the $CD4^+$ Cell Count in HIV +ve subjects

AST	1.00											
ALT	0.70	1.00										
ALP	0.31	0.22	1.00									
ALB	0.12	-0.01	-0.06	1.00								
Bil-T	-0.10	-0.16	-0.05	0.04	1.00							
Bil-D	-0.10	-0.14	-0.07	-0.02	0.77	1.00						
Urea	0.11	0.12	0.10	0.02	-0.07	-0.04	1.00					
Creat	-0.08	-0.12	-0.15	0.20	0.10	-0.03	0.03	1.00				
TC	-0.09	-0.09	0.29	0.07	-0.22	-0.23	0.10	-0.13	1.00			
TG	0.47	0.38	0.32	-0.10	-0.07	-0.10	-0.12	-0.07	-0.11	1.00		
Glu	0.06	0.11	0.15	0.01	-0.13	-0.11	-0.04	-0.24	0.17	0.07	1.00	
$CD4^+$	-0.36	-0.30	-0.31	0.16	-0.13	-0.13	0.15	0.14	0.35	-0.60	-0.09	1.00
	AST	**ALT**	**ALP**	**ALB**	**Bil-T**	**Bil-D**	**Urea**	**Creat**	**TC**	**TG**	**Glu**	**$CD4^+$**

5.2.2.1 Correlation of $CD4^+$ Cell Count with Liver Function Parameters

Among LFTs, the three enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) reveals a negative correlation with $CD4^+$ cell count, i.e. with the decrease in the $CD4^+$ cell count, the activities (concentration) of AST, ALT and ALP increases. ALT and ALP enzymes show moderate correlation with *r* value of -0.30 and -0.31 respectively while AST enzyme shows a marginally high *r* value of -0.36 as compared to ALT and ALP. Another LFT, albumin (ALB) reveals a positive correlation with *r* value of 0.16 which depicts a weak relationship. Last two LFTs, bilirubin total (Bil-T) and bilirubin direct (Bil-D) reveals a negative correlation with *r* value of -0.13 for each which depicts a weak relationship.

The results of correlation (in terms of positive and negative relationship) of LFTs with CD4 cell count are in conformity with the trends shown by mean concentration comparison of normal and HIV subjects in the respective LFTs (shown in previous section in table 5.1 and 5.2). In both the analysis an increase in concentration of AST, ALT, ALP, Bil-T and Bil-D results in the decrease in $CD4^+$

cell count and hence the continued deterioration of immune functions which may leads to AIDS.

5.2.2.2 Correlation of $CD4^+$ Cell Count with Kidney Function Parameters

The two parameters for kidney function; blood urea and serum creatinine (Creat) reveals a positive correlation with $CD4^+$ cell count. Both blood urea and serum creatinine show very similar *r* values of 0.15 and 0.14 respectively which depicts a weak relationship.

The results of correlation (in terms of positive and negative relationship) of serum creatinine with $CD4^+$ cell count are in conformity with the trends shown by mean concentration comparison of normal and HIV subjects (shown in previous section in table 5.10 and 5.11). The same trend was followed by blood urea in Punjab (State – 1) but marginally reversed in case of Rajasthan (State – 2) where the blood urea level increases marginally with decrease in $CD4^+$ cell count (table 5.11).

5.2.2.3 Correlation of $CD4^+$ Cell Count with Lipid Function Parameters

The two parameters for lipid function; total serum cholesterol (TC) and serum triglyceride (TG) reveals a moderate to strong correlation with $CD4^+$ cell count. TC shows a positive correlation with *r* value of 0.35 which depicts a moderate relationship between its mean concentration and $CD4^+$ cell count. The TG on the other hand shows a negative correlation with *r* value of 0.60 which depicts a strong relationship between its mean concentration and $CD4^+$ cell count.

The results of correlation (in terms of positive and negative relationship) of lipid function tests with CD4 cell count are in conformity with the trends shown by mean concentration comparison of normal and HIV subjects in the respective lipid function tests (shown in previous section in table 5.18 and 5.19). In both the analysis an increase in concentration of TG results in the decrease in $CD4^+$ cell count and hence the continued deterioration of immune functions which may leads to AIDS. The higher *r* value of 0.60 reveals a clear and strong relationship between the two variables. Likewise a decrease in concentration of TG results in the decrease in $CD4^+$ cell count and hence the continued deterioration of immune functions which may leads to AIDS.

5.2.2.4 Correlation of $CD4^+$ Cell Count with Blood Glucose

The random blood glucose (Glu) function reveals a negative correlation with $CD4^+$ cell count (*r* value; -0.09) which depicts a weak and somewhat questionable relationship.

5.2.3 Non Linear Regression Analysis [Empirical Correlation]

To predict the $CD4^+$ cell count by using biochemical parameters, a non linear regression equation was developed using sigma plot software. Initially while trying to develop a correlation between six liver function parameters and $CD4^+$ cell count, the results were quite erroneous. Therefore we developed separate correlations and hence non-linear equations for each specific group (AST-ALT, ALP, TG-TC and Urea-Creatinine), since they all have a direct correlation with $CD4^+$ cell count.

A general non linear regression equation for correlation between $CD4^+$ cell count and any two biochemical parameter can be developed as follows:

$$f = a * x^b * y^c$$

Where f = Predicted CD4+ cell count,

a, b and c are constants to be developed using non linear regression analysis, and

x and y = the biochemical parameters, with which the correlation is being developed.

Out of 148 HIV samples analysed, about ≤ 5% in each of the above regression have strayed out of the standard deviation limit. They have not been considered for regression analysis. This behavior may arise due to some technical error in the concerned parameter analysed. The empirical correlation equations developed for prediction of $CD4^+$ cell count with the help of AST-ALT (equation 5.1), ALP (equation 5.2), TC-TG (equation 5.3) and Urea-Creatinine (equation 5.4) parameters are shown as follows:

$CD4^{+} = 782.3155 * (AST)^{-0.1575} * (ALT)^{-0.0587}$ ---------- Equation 5.1.

$CD4^{+} = 727.2305 * (ALP)^{-0.1295}$ ---------- Equation 5.2.

$CD4^{+} = 741.7724 * (TC)^{-0.2439} * (TG)^{-0.1199}$ ---------- Equation 5.3.

$CD4^{+} = 329.9807 * (Urea)^{0.0356} * (Creatinine)^{0.0402}$ ---------- Equation 5.4.

Table 5.36 shows statistical parameters used for developing empirical correlation equations (Equation 5.1, 5.2, 5.3 and 5.4)

Table 5.36 Statistical Parameters for Non Linear Regression Analysis

Equation No.	Coefficient	*t*	*p* (less than)	*F*	R^2	% Error Band
5.1	a = 782.3155	5.5840	< 0.0001	9.5797	0.93	+ 20.9 to –21.9
	b = -0.1575	-2.5111	< 0.0131			
	c = -0.0587	-0.8857	< 0.3773			
5.2	a = 727.2305	5.0948	< 0.0001	12.5119	0.91	+ 22.9 to – 23.4
	b = -0.1295	-3.4420	< 0.0008			
5.3	a = 741.7724	4.6578	< 0.0001	56.4440	0.94	+ 19.2 to – 21.4
	b = -0.2439	-8.7923	< 0.0001			
	c = 0.1199	4.3375	< 0.0001			
5.4	a = 329.9807	14.5041	< 0.0001	2.4489	0.92	+ 23.7 to – 19.0
	b = 0.0356	1.6718	< 0.0967			
	c =0.0402	1.4186	< 0.1582			

The results for equation 5.1 shows that it hold true within the % age error band of + 20.9 to –21.9 % for predicting $CD4^{+}$ cell count. The R^2 value of 0.93 for the same equation was also above average. The results for equation 5.2 shows that it also hold true within the % age error band of + 22.9 to – 23.4 % for predicting $CD4^{+}$ cell count. The R^2 value of 0.91 for the same equation was also above average. Equation 5.2 was the only equation formed by using only one parameter (ALP) for predicting the $CD4^{+}$ cell count, while rest all equations employ two parameters each. The results for equation 5.3 shows that it hold true within the % age error band of + 19.2 to – 21.4 % for predicting $CD4^{+}$ cell count. The R^2 value of 0.94 for the same equation was found

to be maximum among all equations. The results for equation 5.4 shows that it hold true within the % age error band of + 23.7 to – 19.0 % for predicting $CD4^+$ cell count. The R^2 value of 0.92 for the same equation was also above average. Equation 5.4 which employs kidney function parameters (urea and creatinine) for developing equation gives much better results in terms of R^2 value. On the contrary the same parameters did not yield good correlation with $CD4^+$ cell count (table 5.35). This could be because of the resultant values of kidney function parameters which may fit well in a non linear regression equation yielding a better predictive estimate of $CD4^+$ cell count.

The graphical representation in form of parity plots for the predictions performed by each of the equations is shown in the following figures below (figures 5.12 to 5.15).

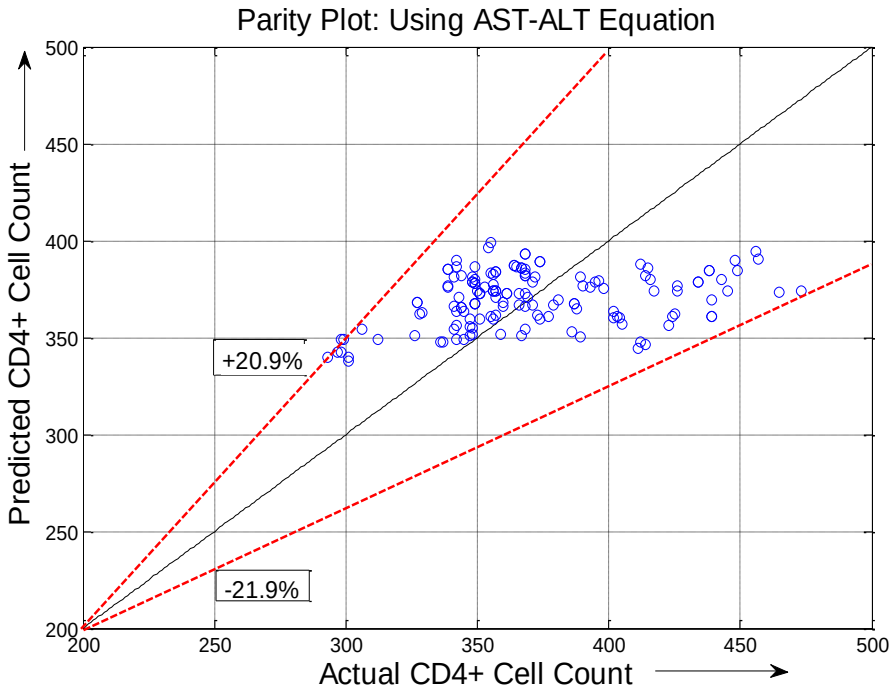

Figure 5.12 Parity Plot: Actual $CD4^+$ versus Predicted $CD4^+$ using AST-ALT Equation

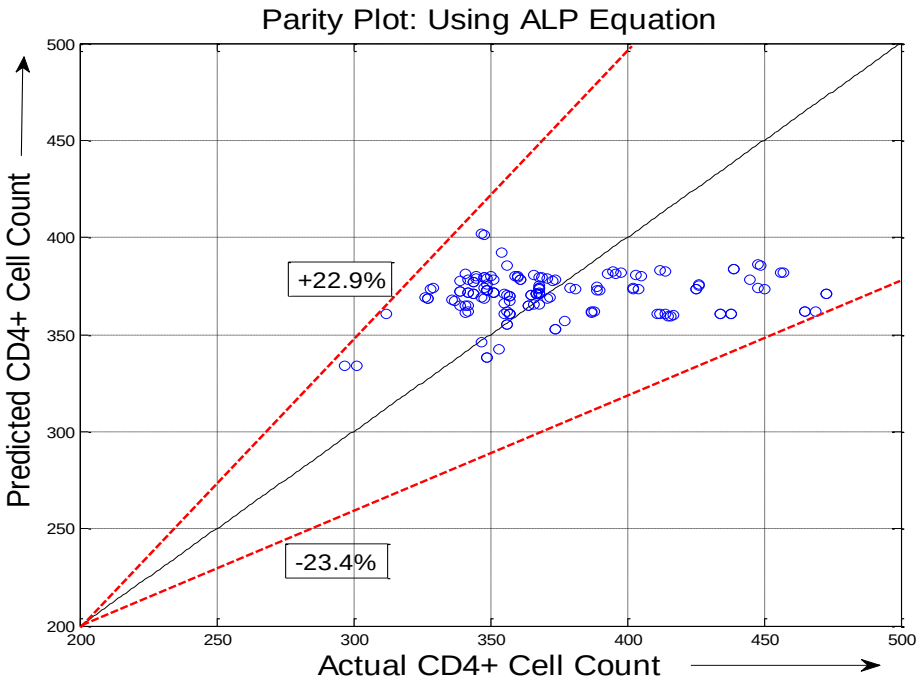

Figure 5.13 Parity Plot: Actual $CD4^+$ versus Predicted $CD4^+$ using ALP Equation

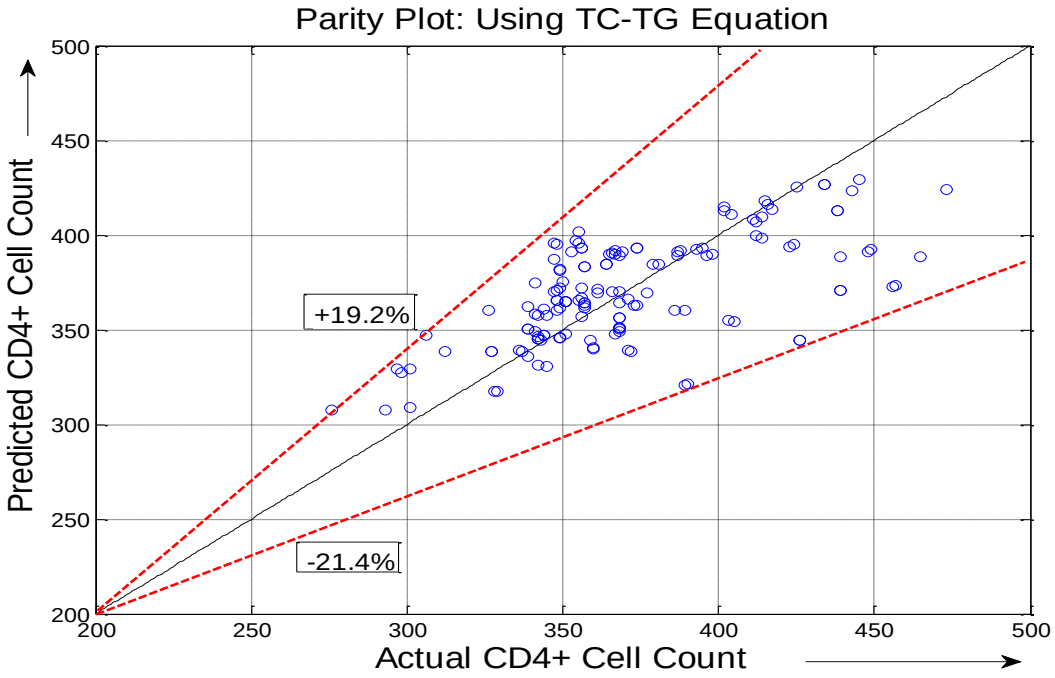

Figure 5.14 Parity Plot: Actual $CD4^+$ versus Predicted $CD4^+$ using TC-TG Equation

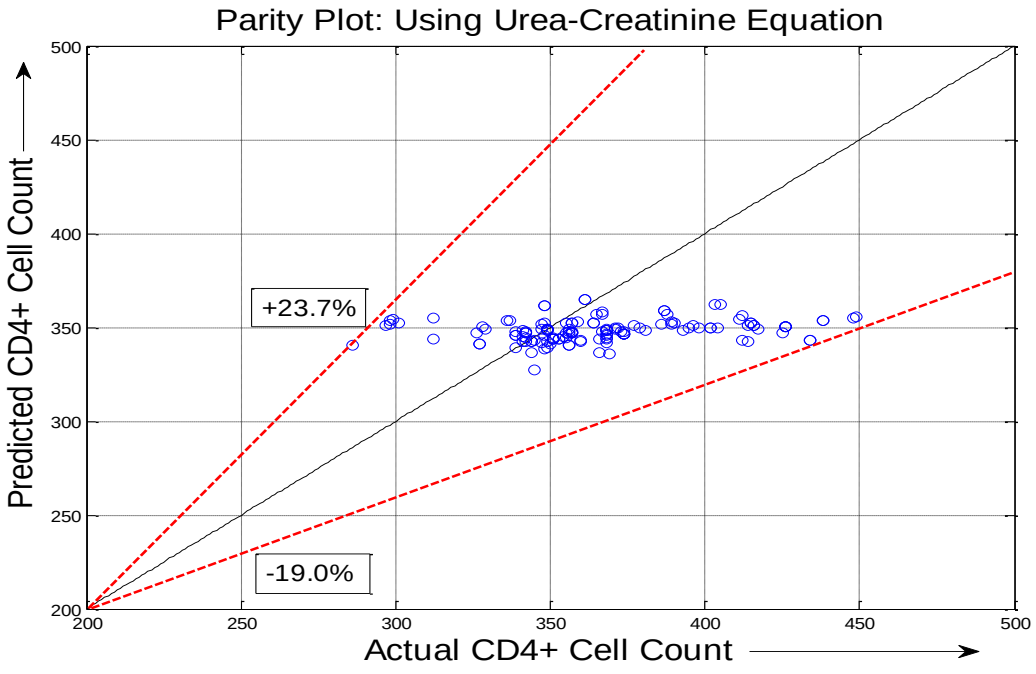

Figure 5.15 Parity Plot: Actual $CD4^+$ versus Predicted $CD4^+$ using Urea-Creat Equation

The $CD4^+$ cell count is now well established to be significantly correlated with AIDS related illness [134, 135, 144]. $CD4^+$ T cell responses in early HIV-1 infection have been found to be important in determining the course of disease progression [225]. The $CD4^+$ cell count done in the present investigation was found to be less than 200 cells/µl in AIDS expressed subjects which is in agreement with Brumme et al. [135] and $CD4^+$ cell count of >200 to <500 / µl is lower than the $CD4^+$ cell count of 679 ± 193/ µl among the healthy Indian subjects [144]. Further the low $CD4^+$ cell count in HIV +ve subjects is associated with liver [225, 226] and kidney [227, 228] dysfunctions as well as with abnormalities in serum lipids (TC and TG) profile and blood sugar [124, 125, 130, 214, 218, 224].

The results of correlation analysis of LFTs with $CD4^+$ cell count are in conformity with the earlier reports [225] to the fact that an increase in AST, ALT, ALP, Bil-T and Bil-D results in simultaneous decrease in $CD4^+$ cell counts. Similarly the kidney function parameters (blood urea and serum creatinine) reveal a parameter specific correlation with the $CD4^+$ cell counts. The observations are in agreement with Jaroszewicz et al. [227]. An increase in TG level and decrease in TC concentration with simultaneous decrease in $CD4^+$ cell count in both cases finds a strong correlation.

Earlier applications of algorithm have been attempted to use total lymphocyte count and hemoglobin profile of subject to predict $CD4^+$ cell count [166, 229-232]. However no report is known to this author about prediction of $CD4^+$ cell count using other biochemical parameters. When non linear regression analysis was performed to find the correlation between the various biochemical parameters and $CD4^+$ cell count, the multivariate regression does not led to desirable results. However, the liver function, kidney function and lipid profile are separately correlated with $CD4^+$ cell count, by using the non linear regression equation; $f = a * x^b * y^c$. The results of the analysis reveal that from the biochemical data, tentative $CD4^+$ cell count obtained were in statistically permissible error limits of the actual $CD4^+$ cell count.

Therefore it may be possible that if the $CD4^+$ cell count is not available in a laboratory, a tentative $CD4^+$ cell count can be calculated separately each for respective LFT, KFT and lipid profile. If the count comes out to be more or less similar in all the cases, the person may be considered exposed to HIV, and if not, then the subject may

be normal. However more work is required to authenticate this method of $CD4^+$ cell count prediction.

5.3 Molecular Marker Analysis

In the molecular marker analysis, amplification with random primers was performed for assessing phylogenetic relationship between subjects of two populations studied and also to determine possible primer sequences for HIV differentiation. In the present analysis, we randomly chose genotypes from 44 HIV +ve human subjects from two different geographical regions—First 22 from Ludhiana region (Punjab state) and rest 22 from Jodhpur region (Rajasthan state) in North India.

5.3.1 Polymerase Chain Reaction (PCR) Amplification

The 44 DNA samples isolated from different HIV +ve subjects from state-1 and state-2 were amplified by 12 different random primers [FA-1 to FA-4, AST-1, AST-2, ALT-1, ALT-2, ALP-1, ALP-2, ALB-1 and ALB-2]. The first four primers (FA-1 to FA-4) were selected from one of the reviewed paper by Aikhionbare et al. [28] on the basis of amplified product size while rest all primers were designed by primer 3D software [29]. The amplified products were analysed using agarose gel electrophoresis.

The genotypes from Punjab state were named (abbreviated) as PJB while those from Rajasthan state were named (abbreviated) as RAJ. The molecular marker analysis was performed by amplification of genes using genomic DNA isolated from 22 HIV +ve blood samples from each state. The amplified DNA products generate bands of different sizes in agarose gel. The first and last lanes in each gel were loaded with 100 bp marker ladder for estimation of size of amplified products. The gel images of all the samples amplified by each of the primers are shown in figures 5.16 to 5.39.

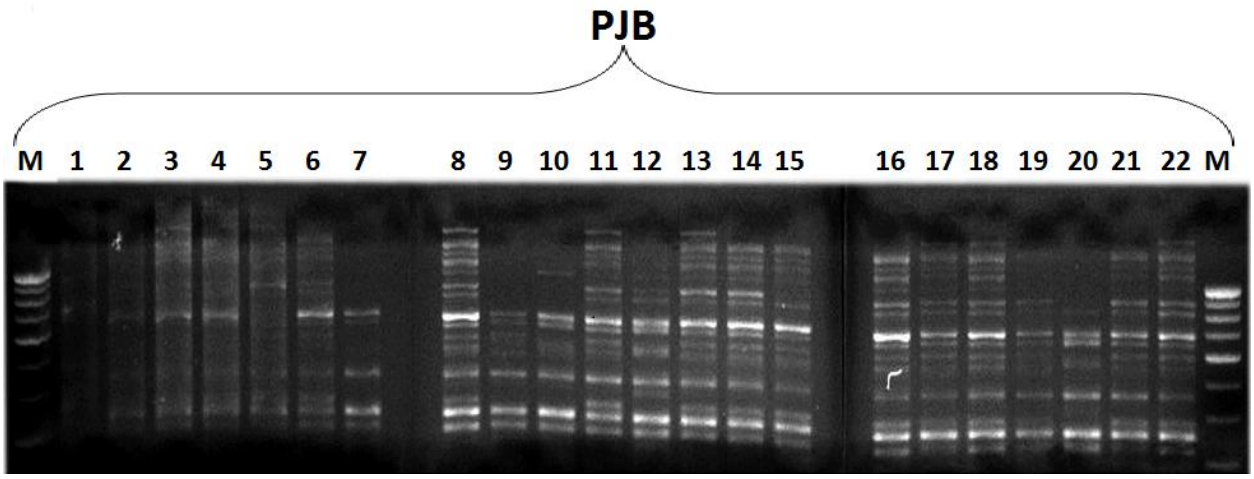

Figure 5.16 Molecular profiling patterns of 22 HIV +ve genomes with FA-1 primer [State-1 (Punjab)]

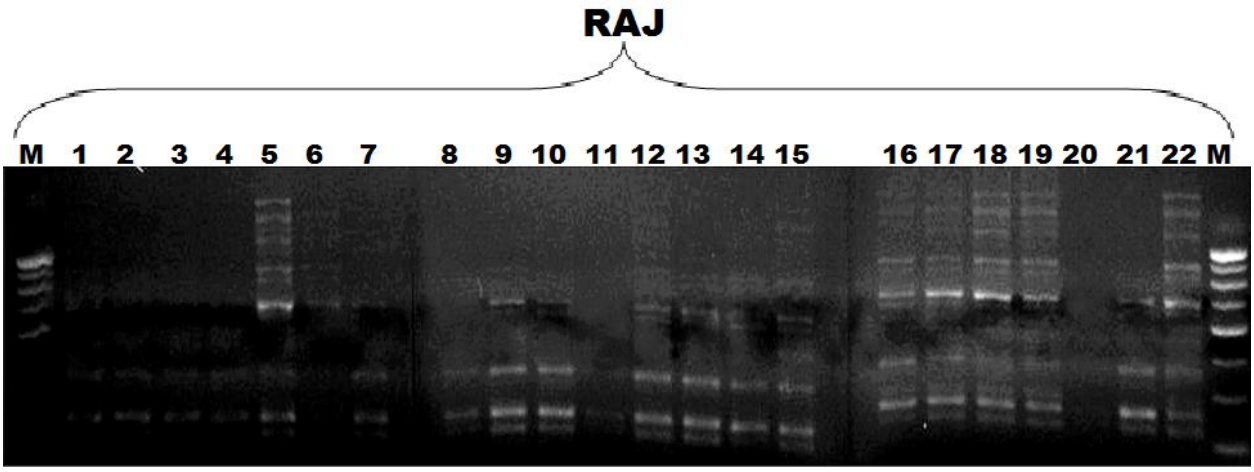

Figure 5.17 Molecular profiling patterns of 22 HIV +ve genomes with FA-1 primer [State-2 (Rajasthan)]

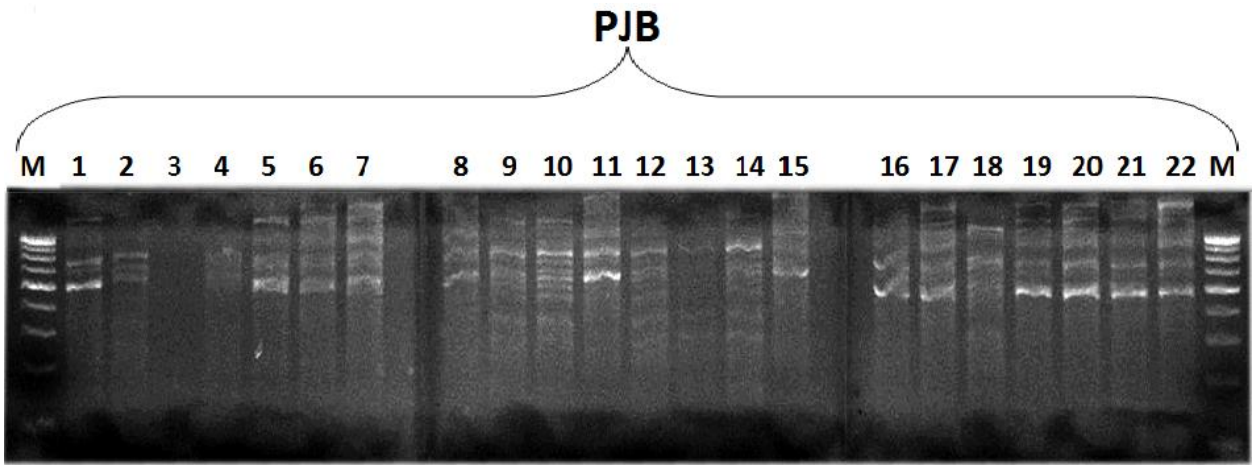

Figure 5.18 Molecular profiling patterns of 22 HIV +ve genomes with FA-2 primer [State-1 (Punjab)]

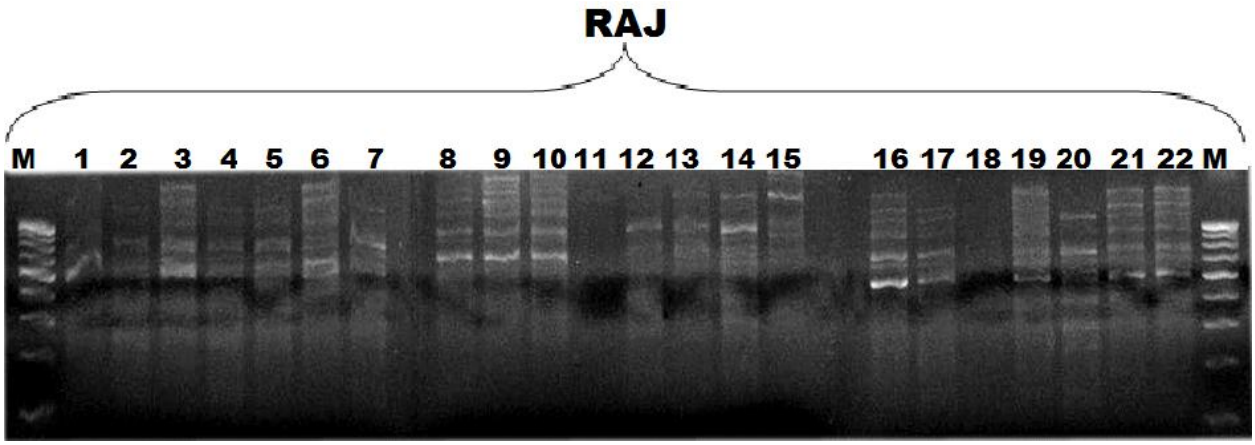

Figure 5.19 Molecular profiling patterns of 22 HIV +ve genomes with FA-2 primer [State-2 (Rajasthan)]

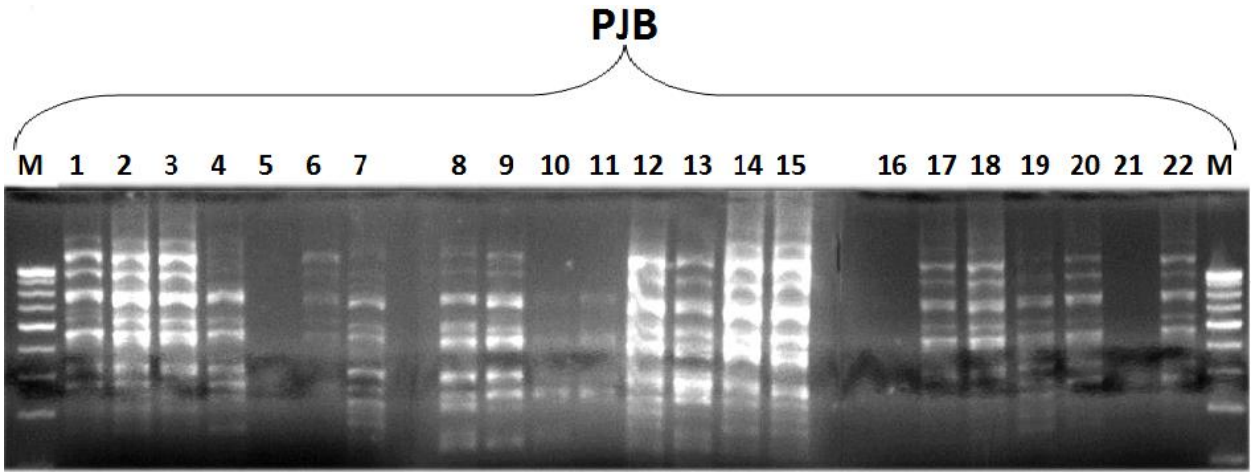

Figure 5.20 Molecular profiling patterns of 22 HIV +ve genomes with FA-3 primer [State-1 (Punjab)]

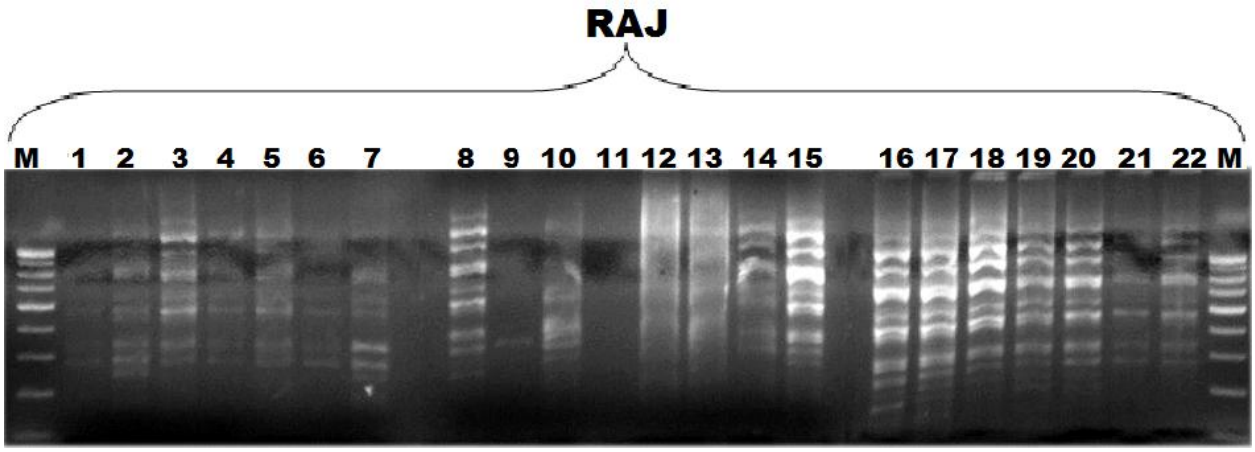

Figure 5.21 Molecular profiling patterns of 22 HIV +ve genomes with FA-3 primer [State-2 (Rajasthan)]

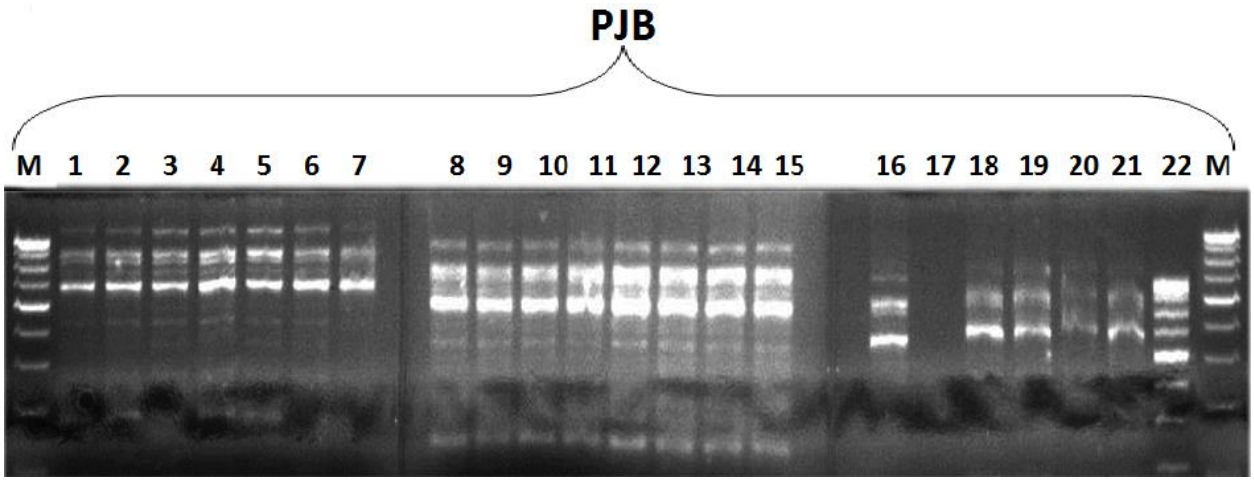

Figure 5.22 Molecular profiling patterns of 22 HIV +ve genomes with FA-4 primer [State-1 (Punjab)]

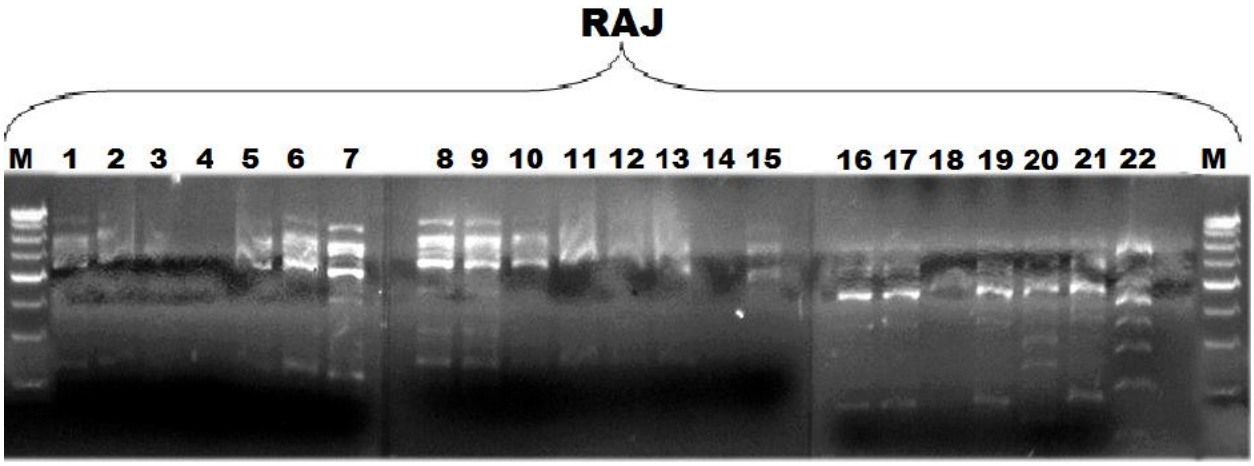

Figure 5.23 Molecular profiling patterns of 22 HIV +ve genomes with FA-4 primer [State-2 (Rajasthan)]

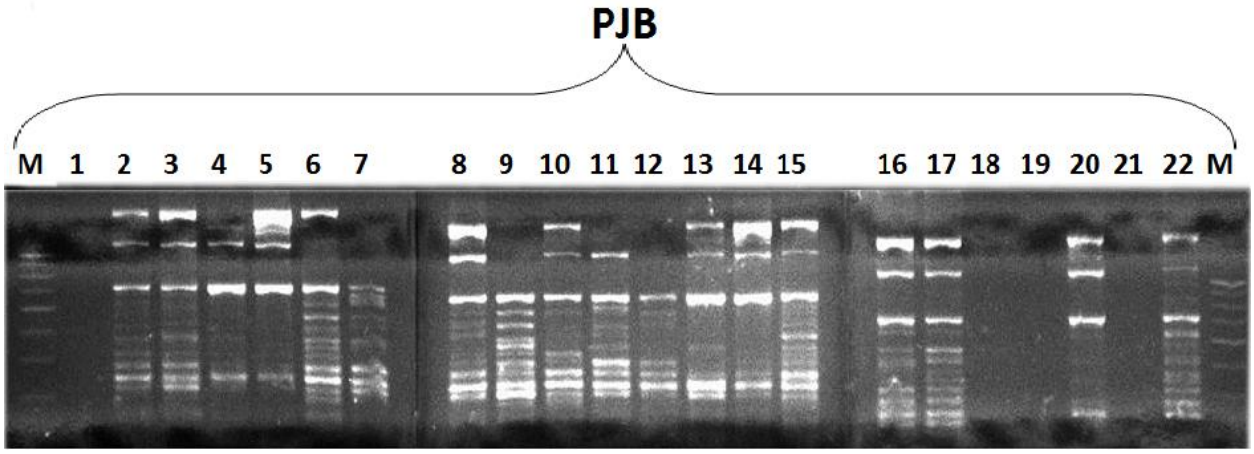

Figure 5.24 Molecular profiling patterns of 22 HIV +ve genomes with AST-1 primer [State-1 (Punjab)]

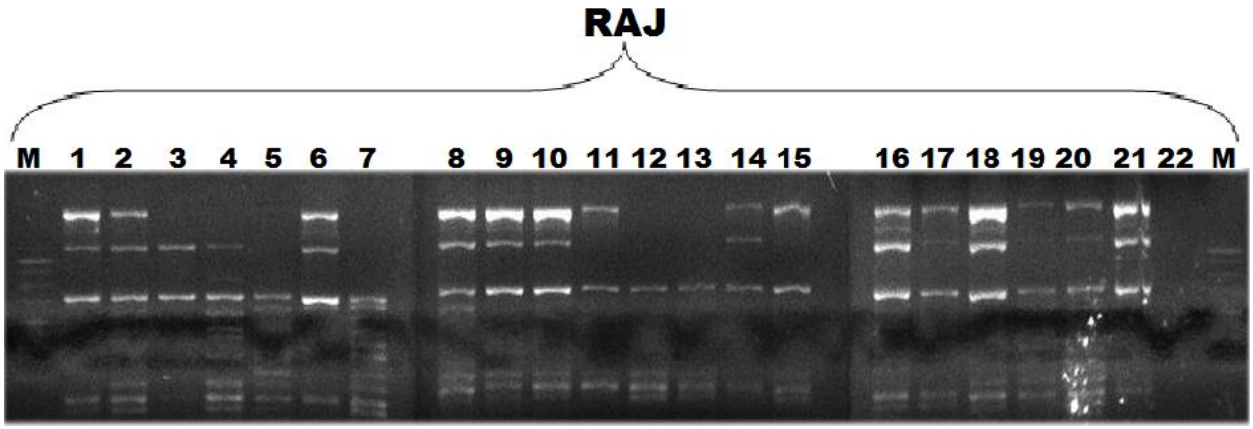

Figure 5.25 Molecular profiling patterns of 22 HIV +ve genomes with AST-1 primer [State-2 (Rajasthan)]

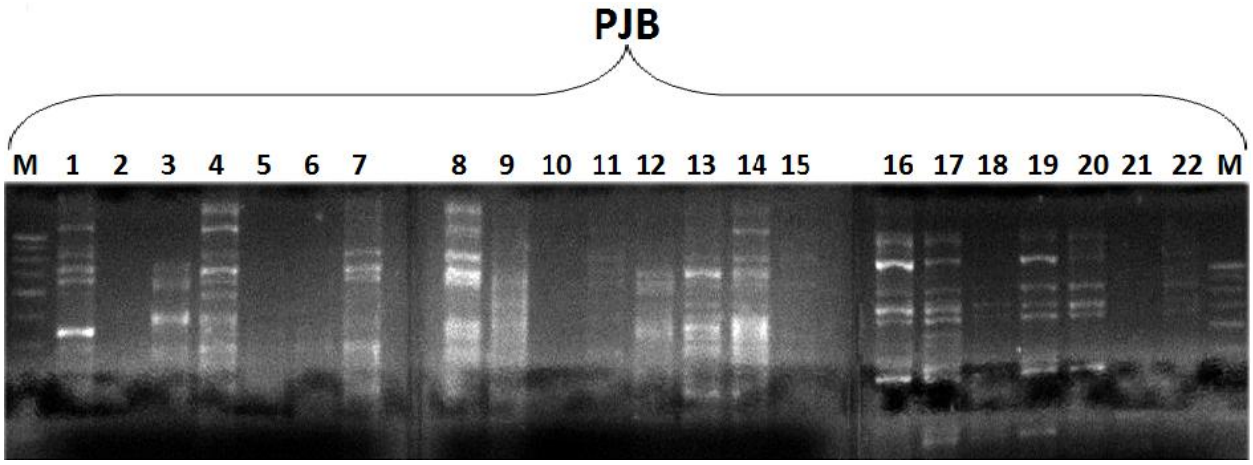

Figure 5.26 Molecular profiling patterns of 22 HIV +ve genomes with AST-2 primer [State-1 (Punjab)]

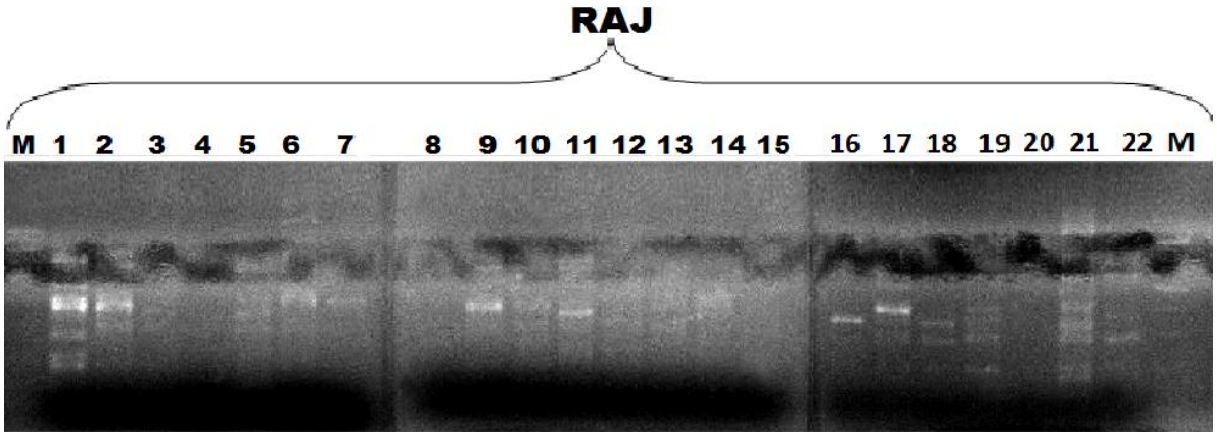

Figure 5.27 Molecular profiling patterns of 22 HIV +ve genomes with AST-2 primer [State-2 (Rajasthan)]

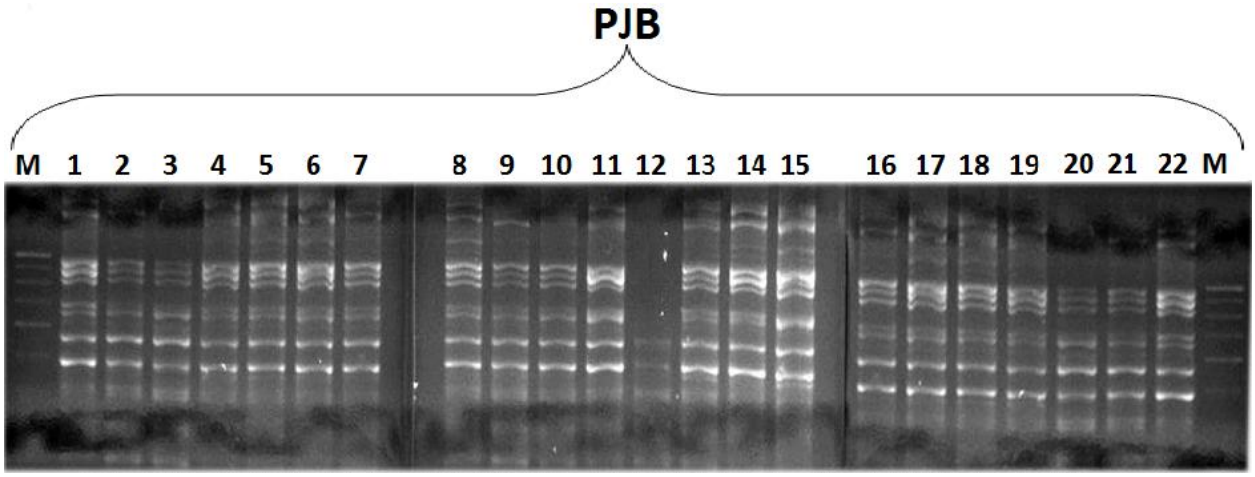

Figure 5.28 Molecular profiling patterns of 22 HIV +ve genomes with ALT-1 primer [State-1 (Punjab)]

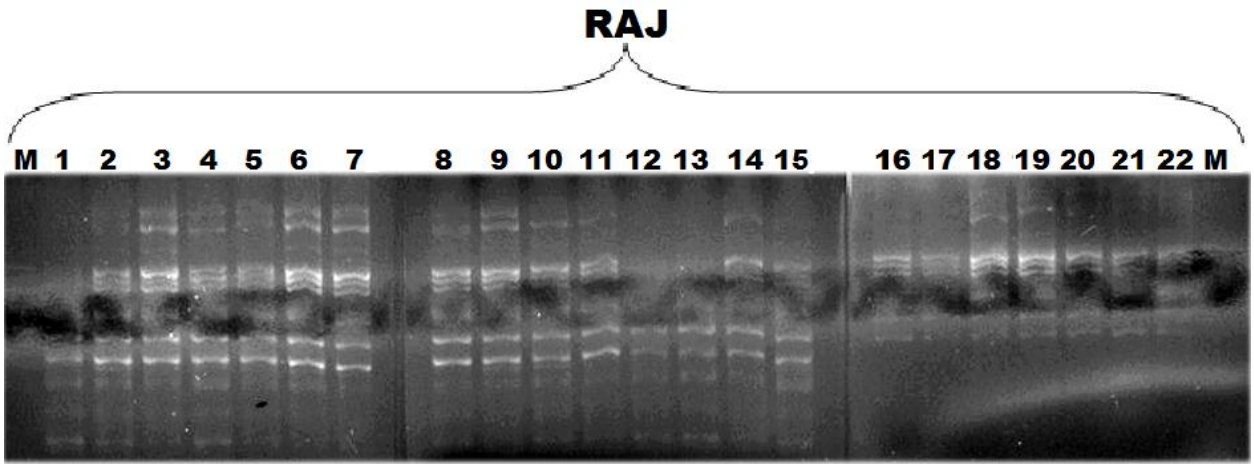

Figure 5.29 Molecular profiling patterns of 22 HIV +ve genomes with ALT-1 primer [State-2 (Rajasthan)]

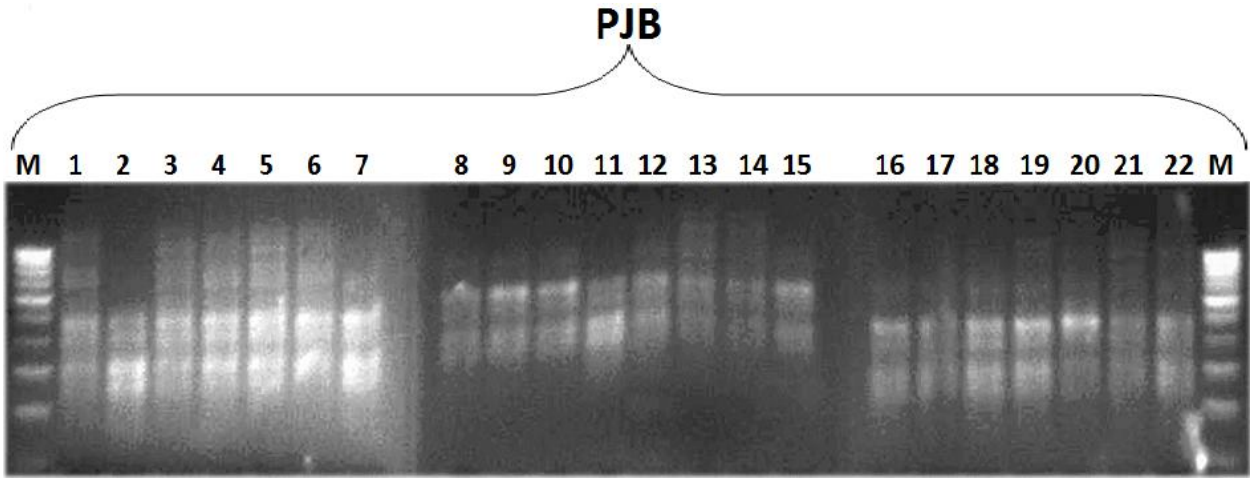

Figure 5.30 Molecular profiling patterns of 22 HIV +ve genomes with ALT-2 primer [State-1 (Punjab)]

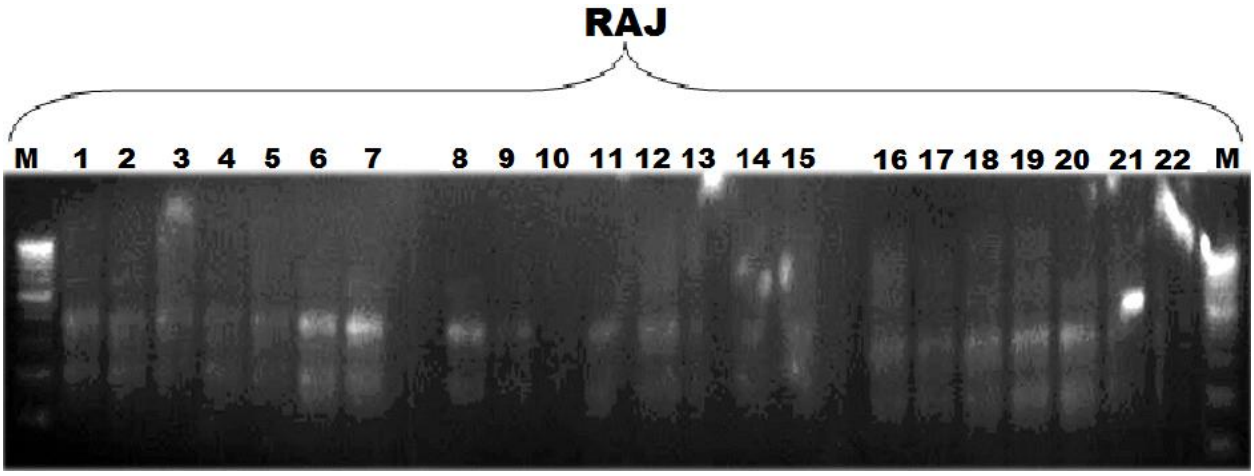

Figure 5.31 Molecular profiling patterns of 22 HIV +ve genomes with ALT-2 primer [State-2 (Rajasthan)]

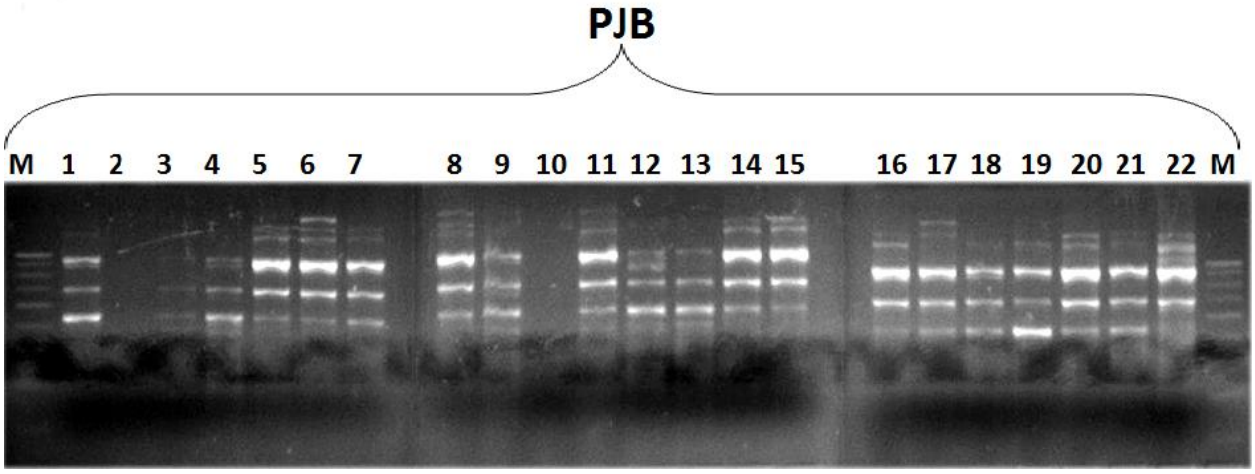

Figure 5.32 Molecular profiling patterns of 22 HIV +ve genomes with ALP-1 primer [State-1 (Punjab)]

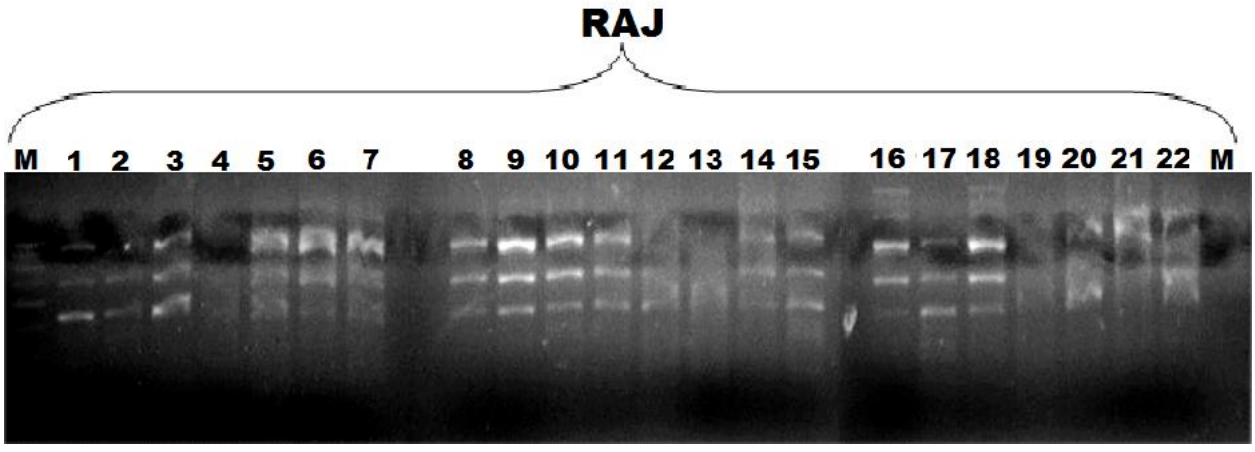

Figure 5.33 Molecular profiling patterns of 22 HIV +ve genomes with ALP-1 primer [State-2 (Rajasthan)]

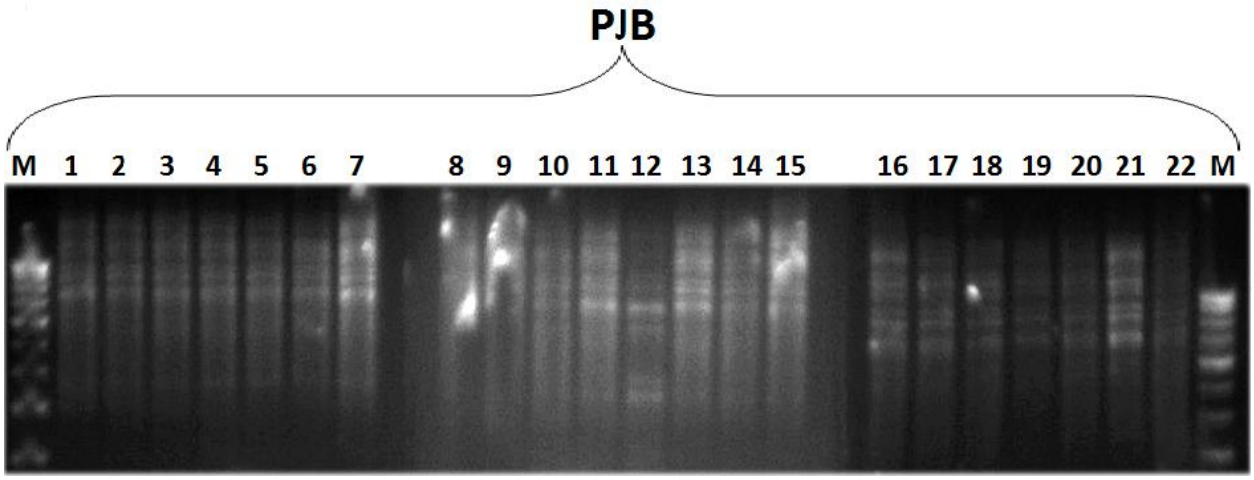

Figure 5.34 Molecular profiling patterns of 22 HIV +ve genomes with ALP-2 primer [State-1 (Punjab)]

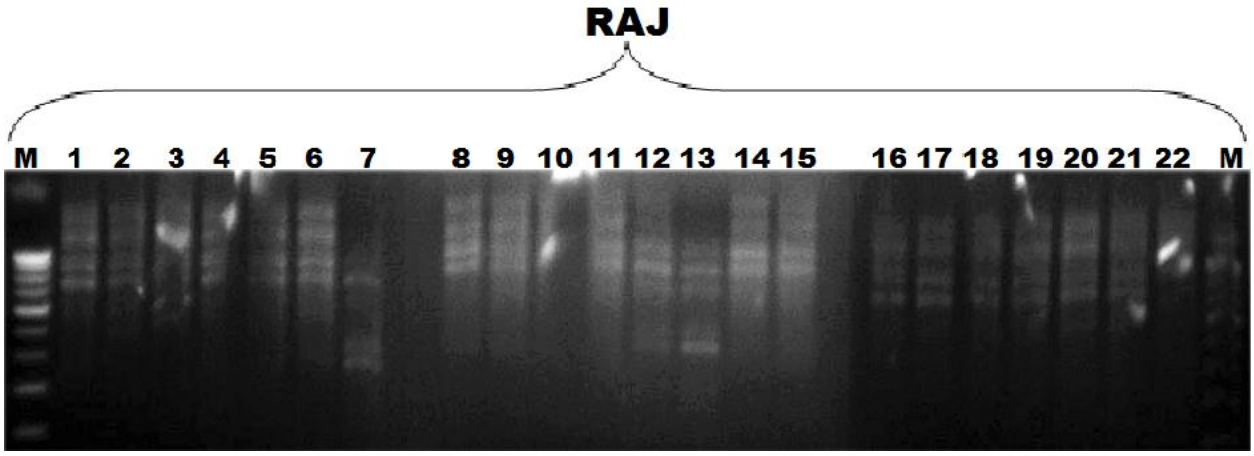

Figure 5.35 Molecular profiling patterns of 22 HIV +ve genomes with ALP-2 primer [State-2 (Rajasthan)]

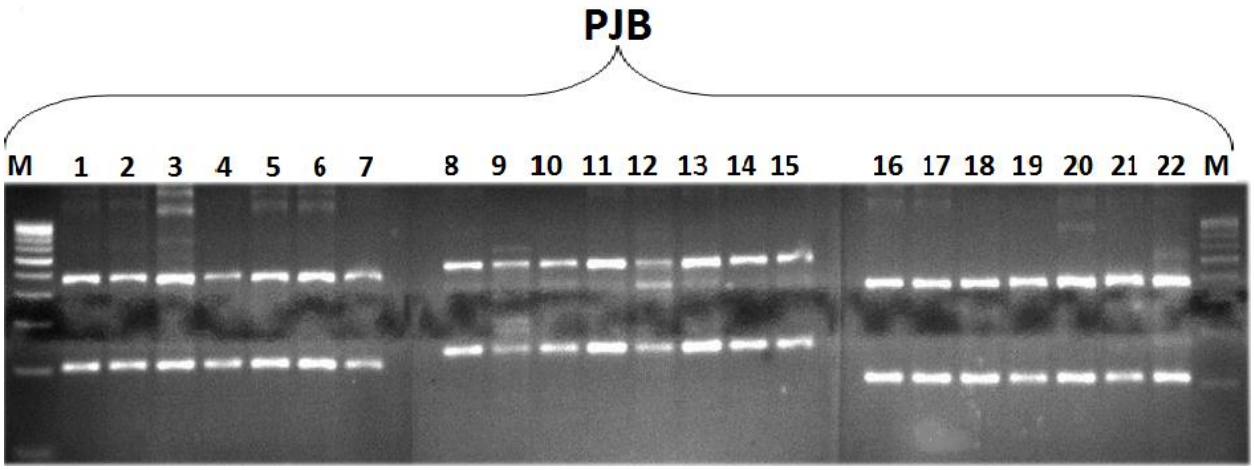

Figure 5.36 Molecular profiling patterns of 22 HIV +ve genomes with ALB-1 primer [State-1 (Punjab)]

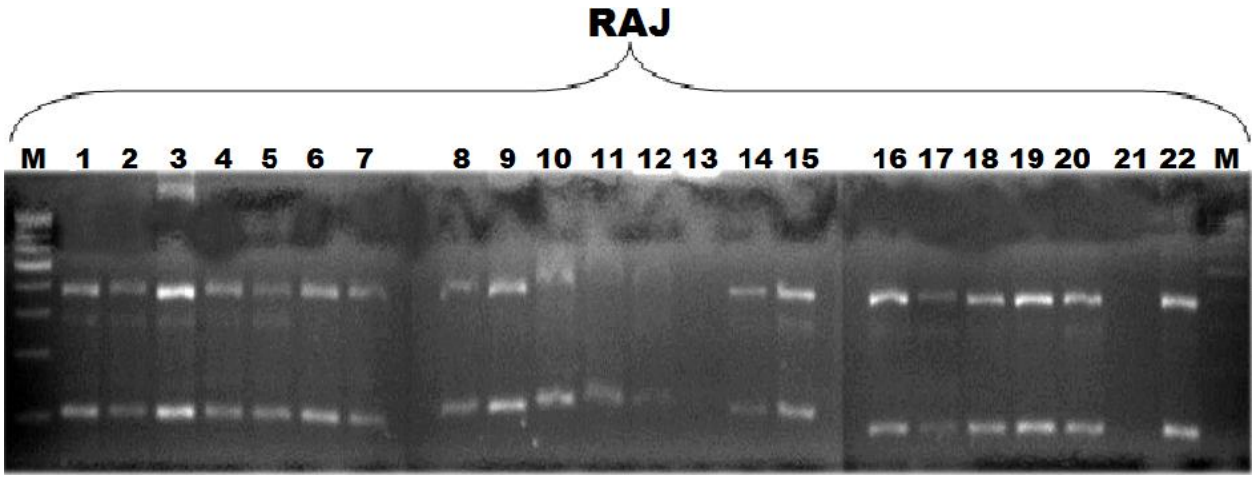

Figure 5.37 Molecular profiling patterns of 22 HIV +ve genomes with ALB-1 primer [State-2 (Rajasthan)]

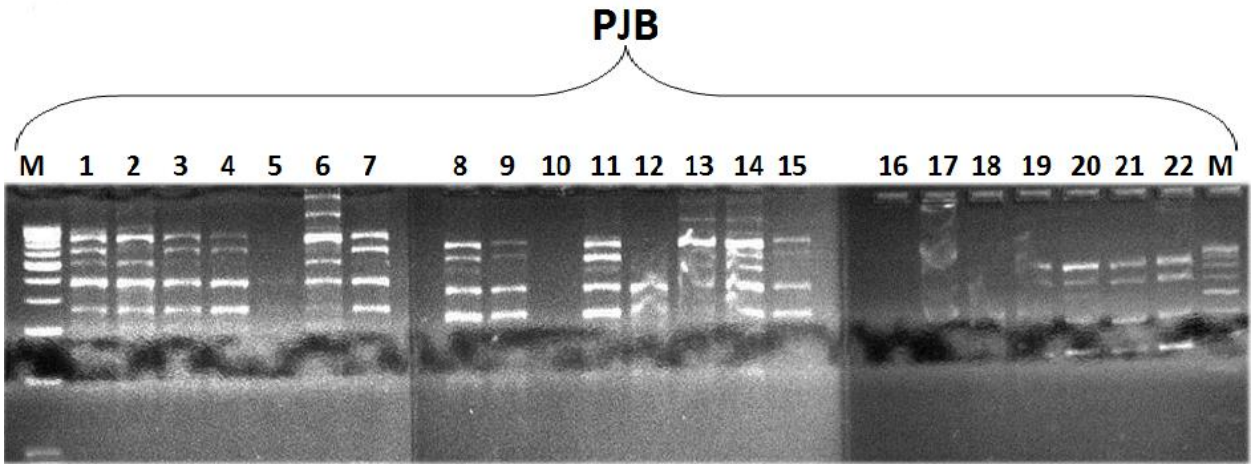

Figure 5.38 Molecular profiling patterns of 22 HIV +ve genomes with ALB-2 primer [State-1 (Punjab)]

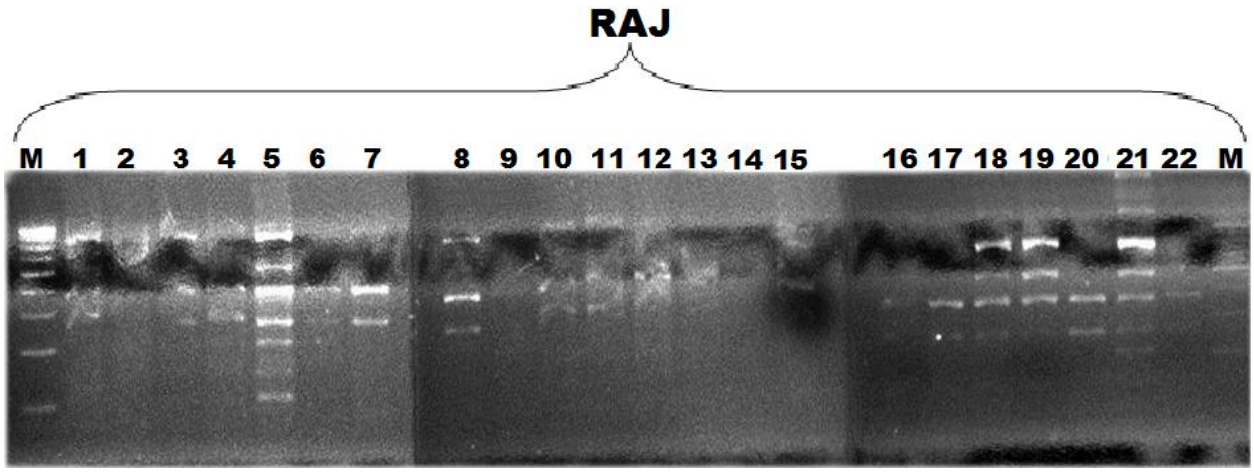

Figure 5.39 Molecular profiling patterns of 22 HIV +ve genomes with ALB-2 primer [State-2 (Rajasthan)]

The results of polymerase chain reaction (PCR) amplification of 44 genotypes by 12 random amplified polymorphic DNA (RAPD) primers are as shown in table 5.37.

Table 5.37 Results of Primer Amplification

S. No.	RAPD Primer	Annealing Temperature (°C)	Molecular weight range (bp)	Number of Polymorphic bands	Number of Monomorphic band	Diversity in value of PIC*	Resolving Power (R_p)
1.	FA-1	31	200 - 1800	9	4	0.4311	1.3787
2.	FA-2	28	200 - 1500	4	3	0.2654	1.4692
3.	FA-3	31	100 - 1600	3	7	0.2065	1.5871
4.	FA-4	33	250 - 1100	2	3	0.2437	1.5126
5.	AST-1	52	200 - 1300	4	5	0.3091	1.3818
6.	AST-2	52	200 - 1400	6	1	0.6415	0.7171
7.	ALT-1	59	300 - 1500	0	9	0	2.0
8.	ALT-2	53	100 - 1200	2	5	0.2480	1.5041
9.	ALP-1	55	300 - 1300	2	4	0.2774	1.4452
10.	ALP-2	53	200 - 2000	3	6	0.2905	1.4191
11.	ALB-1	62	100 - 2200	3	5	0.3620	1.2761
12.	ALB-2	61	150 - 1400	3	4	0.3724	1.2552

* PIC: Polymorphic Information Content

5.3.2 Phylogenetic Analysis

The gel images obtained after PCR amplification were scored using 0-1 analysis. Each band present for a particular genome was referred as 1, while the absence of band was shown by 0. The data was analysed through Numerical Taxonomy and Multivariate Analysis System (NTSYS) software and converted into dendograms as shown in figures 5.40 to 5.44. The dendograms were further analyzed by UPGMA based Jacquard's similarity coefficient. This was done to find out similarity or differences between samples from two geographical regions.

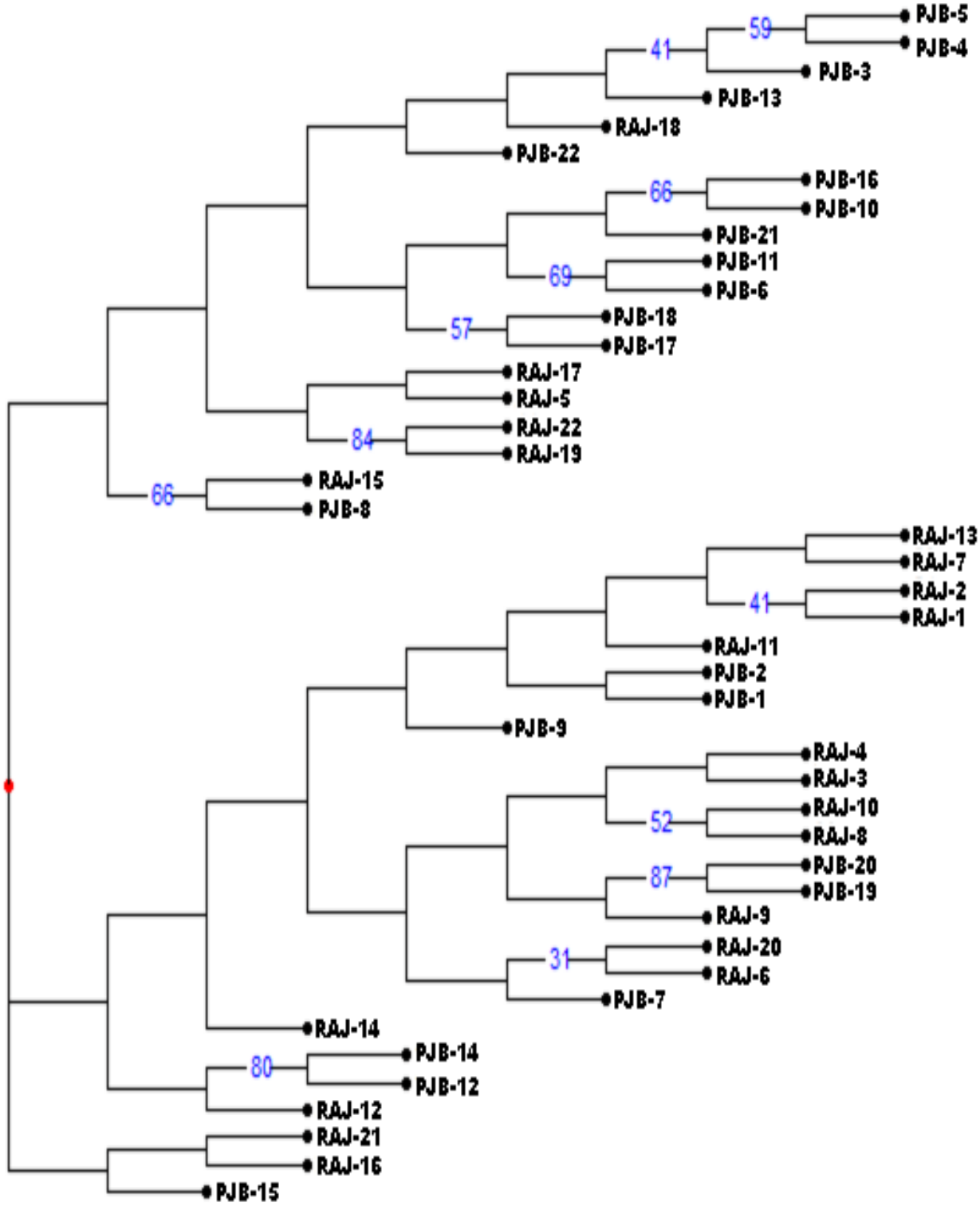

Figure 5.40 Dendogram for RAPD primers 1 – 4

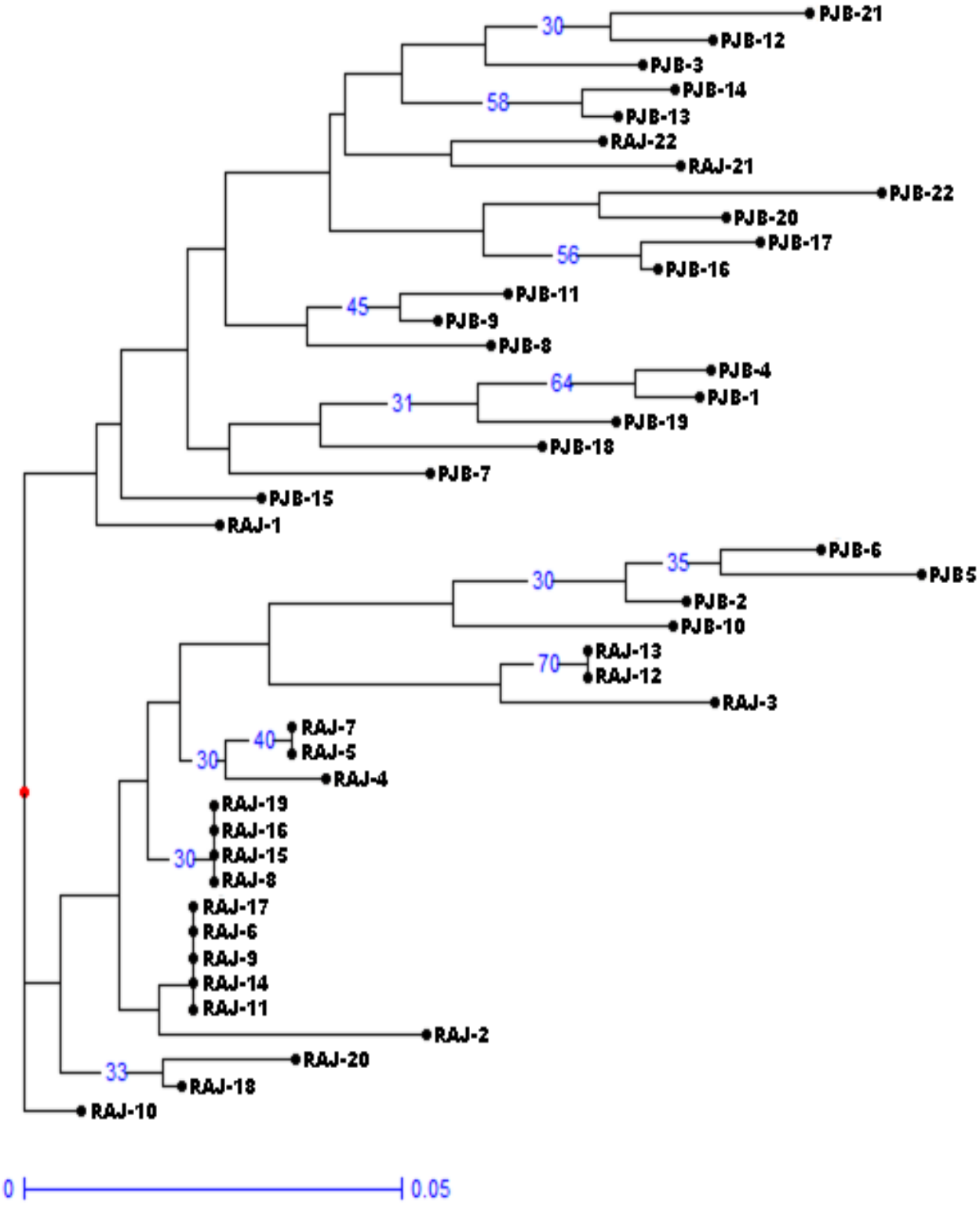

Figure 5.41 Dendogram for primers AST-1, AST-2, ALT-1 & ALT-2

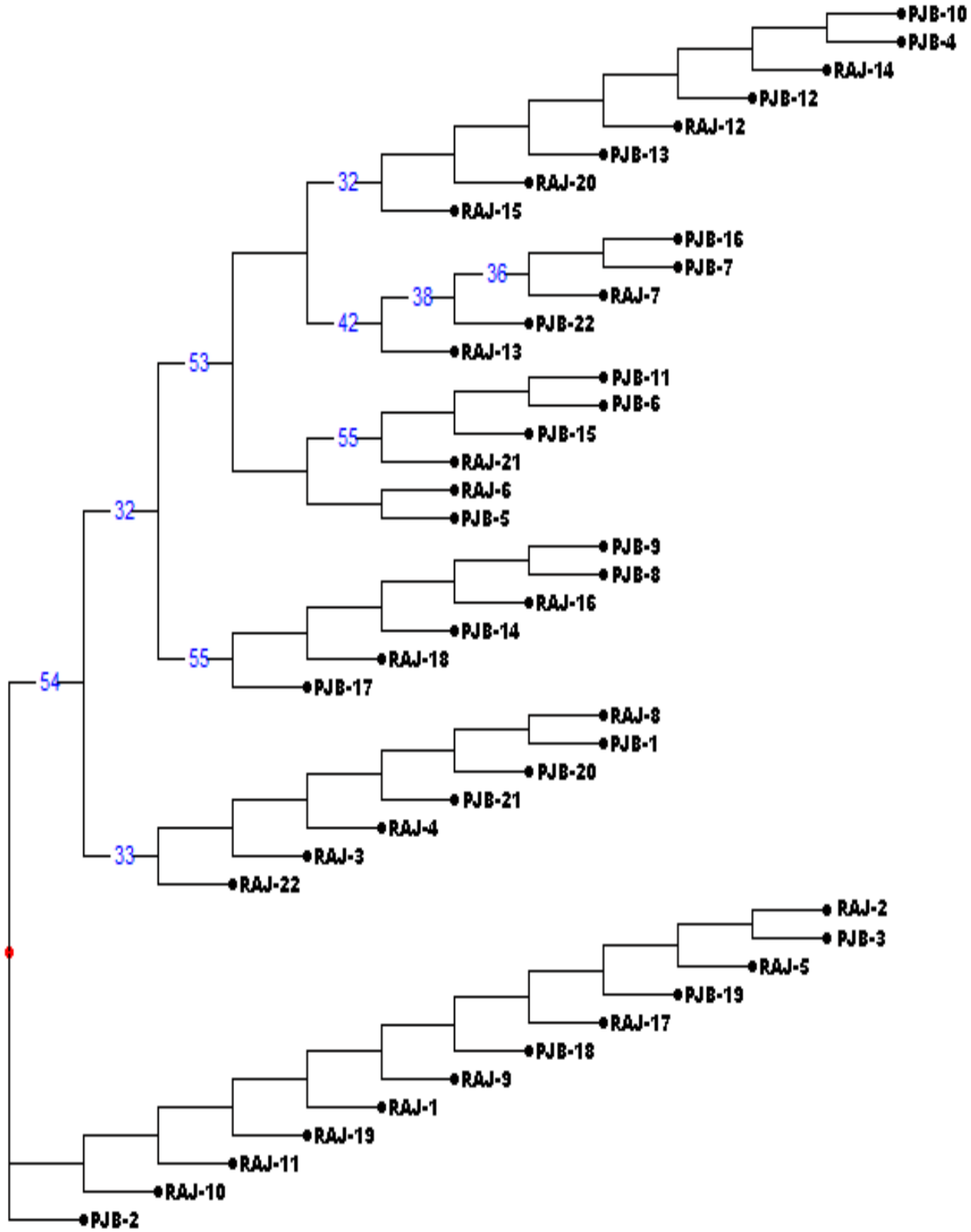

Figure 5.42 Dendogram for primers ALP-1, ALP-2

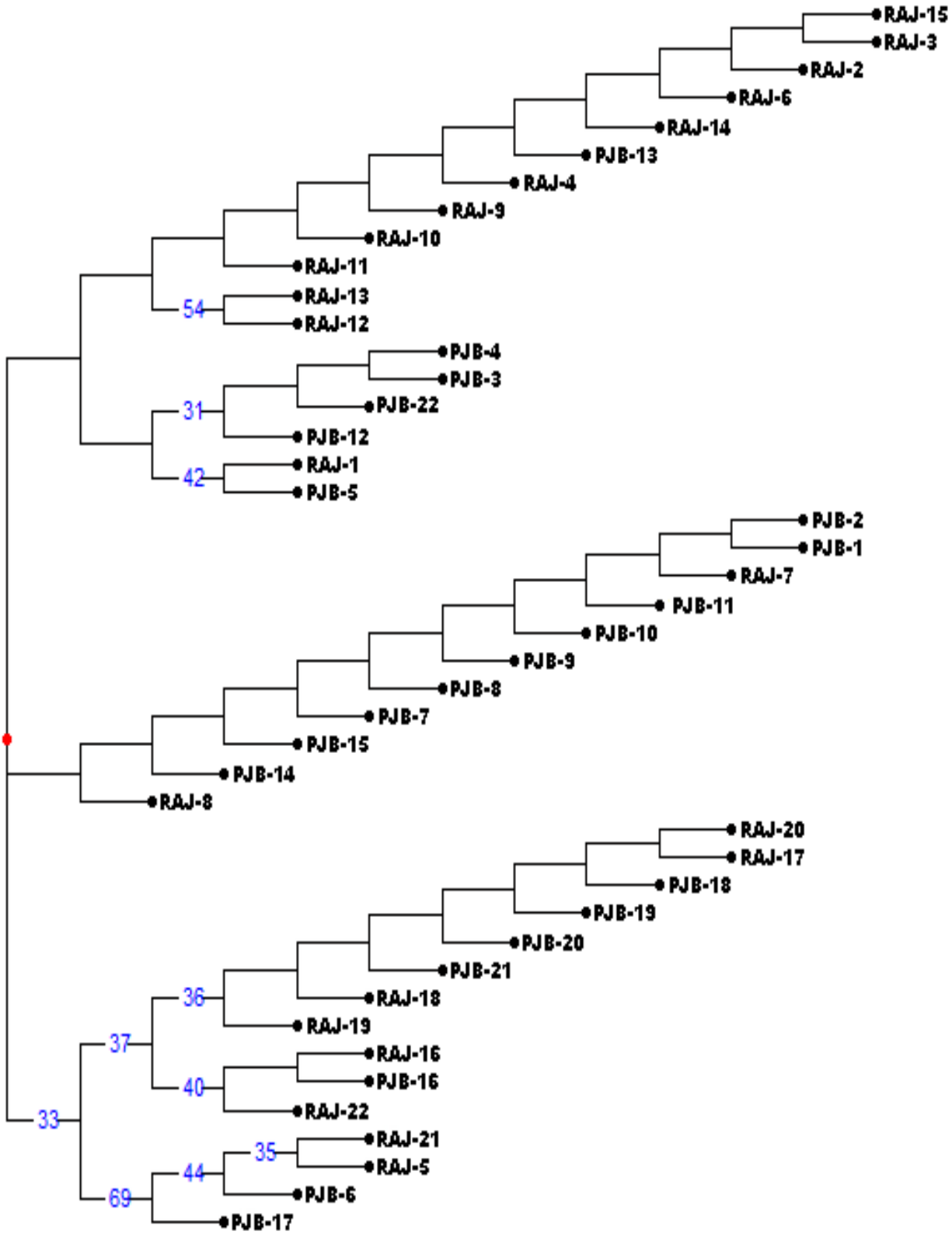

Figure 5.43 Dendogram for primers ALB-1, ALB-2

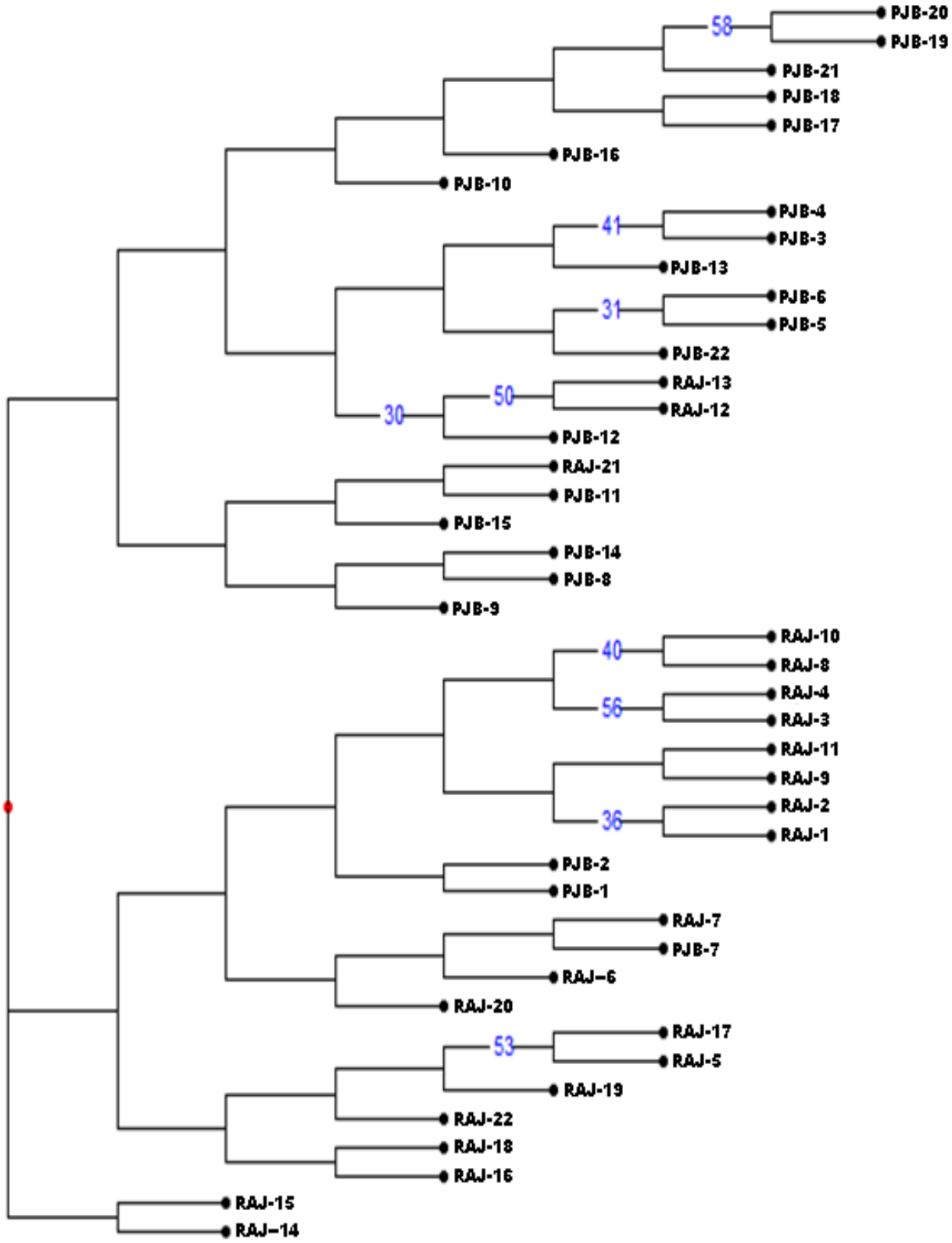

Figure 5.44 Dendogram for all primers (Combined)

The results of dendogram analysis are as discussed below:

5.3.2.1 RAPD Primer Analysis

Figure 5.40 shows the dendogram formed by four randomly chosen primers (FA-1 to FA-4). These four randomly selected RAPD primers divide genotypes into six clusters which were further sub-divided into 15 sub-clusters. As already known, RAPD primers correspond to non-specific regions of the genome. These non-specific regions may be those regions which have continuous expression; that is why RAPD primer set shows similarity between genotype of two different regions. Samples from Punjab state (PJB-1 to PJB-22) were clustered with samples from Rajasthan (RAJ-1 to RAJ-22). For example, PJB-7, RAJ-6 and RAJ-20 were grouped in same cluster. Similarly RAJ-18 is grouped with PJB-22, PJB-13 and other Punjab region's samples. In RAPD primer profiling, genotype PJB-15 form absolutely separate cluster which follows the same pattern as observed in other group of primers (AST-ALT group, ALB group and some what in ALP group). The average polymorphic information content (PIC) and resolving power (R_P) values of RAPD primer set were (0.2867) and (1.4869) respectively.

5.3.2.2 AST-ALT Primer Analysis

Figure 5.41 shows the dendogram formed by two AST and two ALT primers (AST-1, AST-2, ALT-1 and ALT-2). This primer set divides all 44 genotypes into eight sub-clusters, which were further sub-divided into 15 sub-clusters. These primers shows very unique similarity pattern between the genotypes. Genotypes from two different regions were almost grouped in same sub-clusters. On one hand some genotypes, for example RAJ-19, RAJ-16, RAJ-15 and RAJ-8 shows very close relationship with RAJ-17, RAJ-6, RAJ-9, RAJ-14 and RAJ-11. Contrary to these, genotypes like RAJ-10, RAJ-1 and PJB-15 shows very distinct patterns and form almost separate cluster. Similarly, same primer shows different banding pattern and hence different clusters were formed from genotypes of a single region as in RAJ-10. The average polymorphic information content (PIC) and resolving power (R_P) values of AST-ALT primer set were (0.2997) and (1.4008) respectively.

5.3.2.3 ALP Primer Analysis

Figure 5.42 shows the dendogram formed by two ALP primers (ALP-1 and ALP-2). ALP primers divide 44 genotypes into four clusters which were further divided into eight sub-clusters. ALP primers show no characteristic pattern in cluster formation. Samples from two different regions shows similarity with each other with few exceptions for example, genotype PJB-2 forms a separate cluster while PJB-1 shows similarity with RAJ-8 and other Rajasthan region genomes. The average polymorphic information content (PIC) and resolving power (R_P) values of ALP primer set were (0.2840) and (1.4322) respectively.

5.3.2.4 ALB Primer Analysis

Figure 5.43 shows the dendogram formed by two ALB primers (ALB-1 and ALB-2). These two ALB primers (ALB-1 and ALB-2) divide 44 genotypes into six clusters which were further divided into ten sub-clusters. ALB primer did not show any significant genetic difference between the samples of two geographical regions. As samples from Punjab region, PJB-13 clustered with RAJ-14, RAJ-15, RAJ-13 and other Rajasthan region samples. Similarly, PJB-6 clustered with RAJ-21 and RAJ-5. Genotype PJB-17 form separate sub-cluster, similar to as observed in ALP primer analysis. RAJ-8 also clustered separately from other Rajasthan region samples. Here no distinct characteristic similarity pattern is followed between samples from two geographical regions. The average polymorphic information content (PIC) and resolving power (R_P) values of ALB primer set were (0.3672) and (1.2657) respectively.

5.3.2.5 Combined Primer Analysis

Figure 5.44 shows the dendogram formed by all 12 primers used in the present study (FA-1 to FA-4, AST-1, AST-2, ALT-1, ALT-2, ALP-1, ALP-2, ALB-1 and ALB-2). Combined primer profiling of 44 genotypes with the help of all 12 primers follow some of the characteristic profiling pattern of individual primer analysis, as PJB-15 follow the same pattern in individual as well as in combined analysis. PJB-10, PJB-6 and PJB-5 show the same pattern as in AST-ALT group. RAJ-3, RAJ-4, RAJ-1, RAJ-2, RAJ-14 and RAJ-15 follow the pattern as in ALP primer set. PJB-1,

PJB-2, PJB-7 and RAJ-7 show the pattern followed by ALB primer set. These results confirm the authenticity of molecular marker analysis as the cluster pattern generated by combined primer analysis seems to be an extended pattern of analysis based on different individual primer sets.

Primers with high PIC values are useful for estimating relationships between genotypes. Primers with low PIC values on the other hand can be used to analyse chromosome regions of special interest. The PIC value depends on the diversity of the genotype tested [233-235].

The results of hierarchical cluster analysis carried out with the four marker types together reflected those obtained using each marker type separately. The relationships between the genotypes were also clearly demonstrable. The combined use of various marker types allow the tested genotypes to be reliably distinguished, while also providing a clear picture of how they were related. Our results are in good agreement with those previously obtained using markers [28], which provide a good estimation of genetic correlation [236-238]. The greatest variability was observed when genotypes were analyzed using AST-ALT primers. This could be attributed to the high expression of gene responsible for AST and ALT enzymes in HIV +ve genotypes. AST-ALT set of primers showed maximum polymorphism under given set of conditions with respective genomes. RAPD primers also distinguish genotypes under study remarkably [239]. RAPD analysis could be adapted for the rapid and efficient screening of DNA from large cohort groups to identify unknown relationships that may have clinical relevance to pathology of HIV-1 or other diseases.

Furthermore, RAPD analysis may be a primary tool in exploiting single nucleotide polymorphism (SNP) within human genes, both as related to genotype–phenotype correlations and as a tool for linkage and association studies in complex diseases [28]. RAPD primers bind randomly to different parts of the genome, so theoretically they cover the genome uniformly. Williams et al. [152, 240] suggested that all data from a given set of RAPD-PCR profiles are not equally usable as molecular markers and fact that most polymorphic RAPD markers are dominant [241]. In our study, cluster pattern generated by ALP and ALB set of primers

differentiate various HIV affected genotypes effectively. A combination of all primer sets will therefore help to provide whole genome coverage and reduce the errors in genetic similarity estimation based on any one marker system alone.

Also if we assume that by increasing the number of molecular primers, the variance of individual genetic similarity estimation is decreasing, the large number of polymorphic bands obtained in our analysis should provide more precise genetic similarity estimates. **S**ome studies [242] show that most of the RAPD primers tested, mapped to a narrow chromosome region on chromosome.

Thus without a knowledge on the chromosomal location of various products, the variability between the genotypes by RAPD primers may be overestimated and may result in a considerable distortion. The appearance of multiple bands in the molecular profiles (figure 5.16 - 5.39) is possibly due to non specific binding of the primers. Therefore, the RAPD analysis technique is not very reliable as reported by earlier workers [152, 240-242]. From the above analysis and discussion, it may be concluded that RAPD analysis does not correlate significantly with HIV/AIDS condition and thus not useful in HIV/AIDS diagnosis.

5.3.3 Analysis of Molecular Variance (AMOVA)

The analysis of molecular variance (AMOVA) was performed using 0-1 excel data sheet obtained after scoring the gel images of the amplified products using all the four categories of primer sets and one combined study comprising of all 12 primers. AMOVA is used to describe the partitioning of genetic variation among and within groups in which a distance matrix is created with Arlequin software programme. Arlequin partitions the sum of squared deviations from distance matrix into hierarchical variance components, which are tested for significance using permutation tests.

The statistical results of AMOVA using different primer sets and the combined study are shown in table 5.38 and figures 5.45 to 5.49.

Analysis of molecular variance indicated that when FA1-FA4 primers were used, then higher percentage of variation (84 %) was attributed to within the population variation than among the populations variation (16 %) of region under

study (Figure 5.45). The estimated variance within the population (2.94) was higher as compared to among the populations (0.55) as shown in table 5.38. When AST-ALT primer set was used, AMOVA indicated that, higher percentage of variation (79 %) was attributed to within the population variation than among the population variation (21 %) of region under study (Figure 5.46). The estimated variance within the population (2.15) was higher as compared to among the populations (0.59) as shown in table 5.38.

Table 5.38 Statistical Results for AMOVA

Primer sets	Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Est. Var.	*F*
FA1 FA2 FA3 FA4	Among populations	1.000	15.136	0.554	5.141
	Within Population	42.000	123.636	2.944	
AST – 1 AST – 2 ALT – 1 ALT – 2	Among populations	1.000	15.023	0.585	6.987
	Within Population	42.000	90.318	2.150	
ALP – 1 ALP – 2	Among populations	1.000	1.568	0.024	1.508
	Within Population	42.000	43.682	1.040	
ALB – 1 ALB – 2	Among populations	1.000	3.341	0.117	4.419
	Within Population	42.000	31.773	0.756	
Combined	Among populations	1.000	35.068	1.281	5.089
	Within Population	42.000	289.409	6.891	

When ALP primer set was used, AMOVA indicated that, higher percentage of variation (98 %) was attributed to within the population variation than among the populations variation (2 %) of region under study (Figure 5.47). The estimated variance within the population (1.04) was higher as compared to among the populations (0.02) as shown in table 5.38.

AMOVA indicated that when ALB primer set was used, then higher percentage of variation (87 %) was attributed to within the population variation than among the populations variation (13 %) of region under study (Figure 5.48). The estimated variance within the population (0.76) was higher as compared to among the populations (0.12) as shown in table 5.38.

When combined primers were used, AMOVA indicated that, higher percentage of variation (84 %) was attributed to within the population variation than among the populations variation (16 %) of region under study (Figure 5.49). The estimated variance within the population (6.89) was higher as compared to among the populations (1.28) as shown in table 5.38.

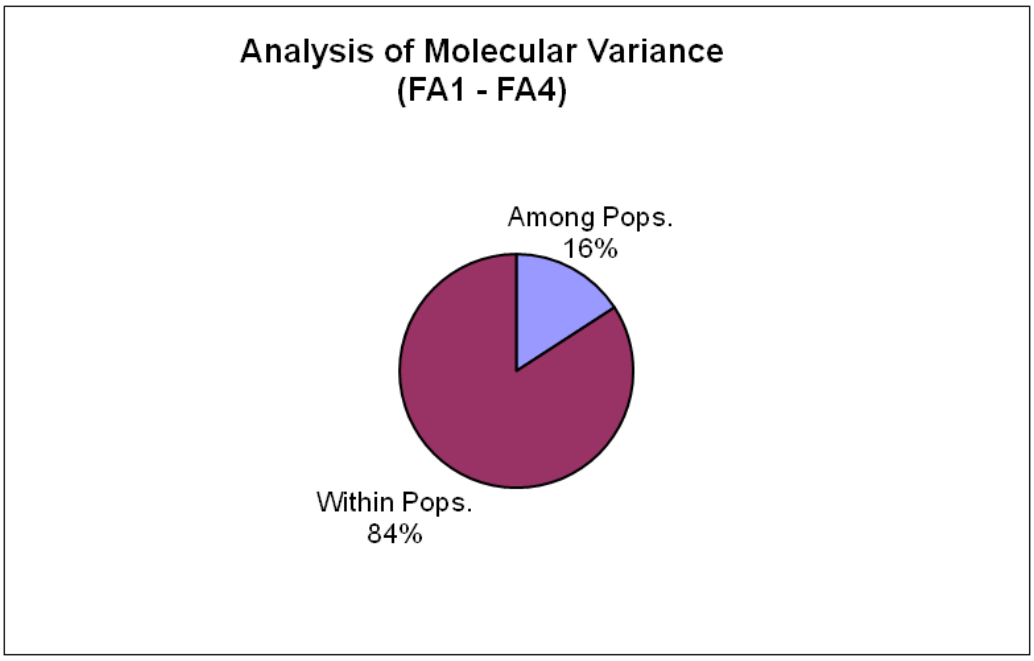

Figure 5.45 Analysis of Molecular Variance (Primer FA1 to FA4)

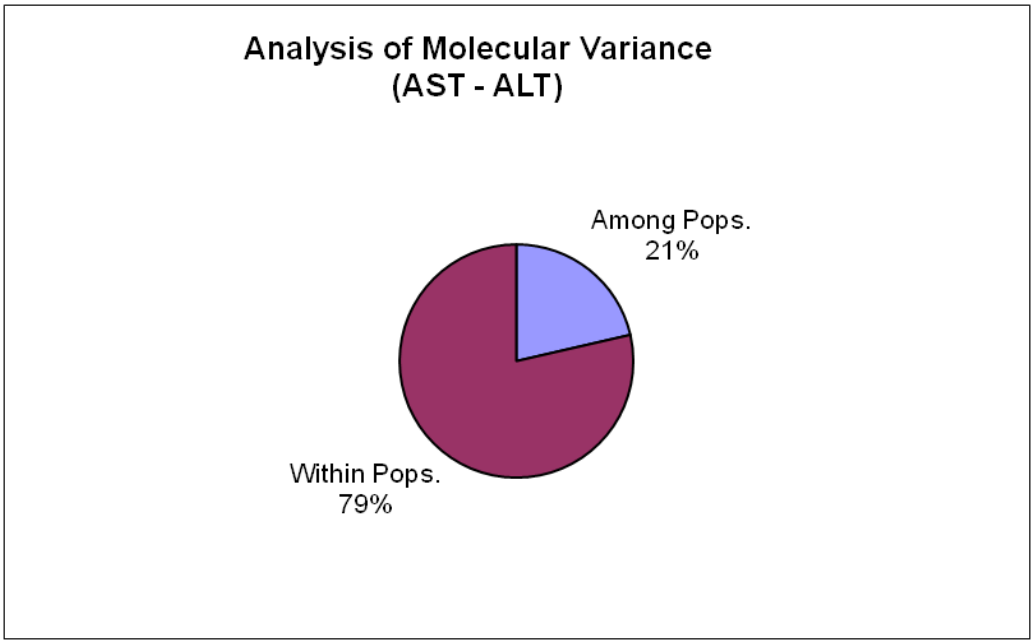

Figure 5.46 Analysis of Molecular Variance (Primer AST-1, AST-2, ALT-1 & ALT-2)

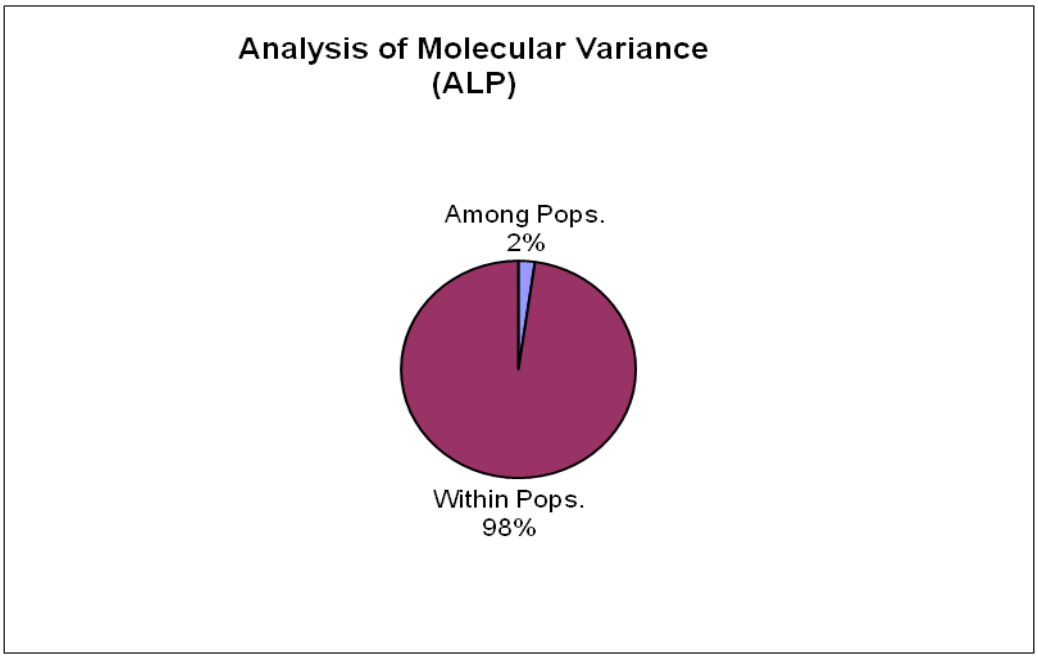

Figure 5.47 Analysis of Molecular Variance (Primer ALP-1 & ALP-2)

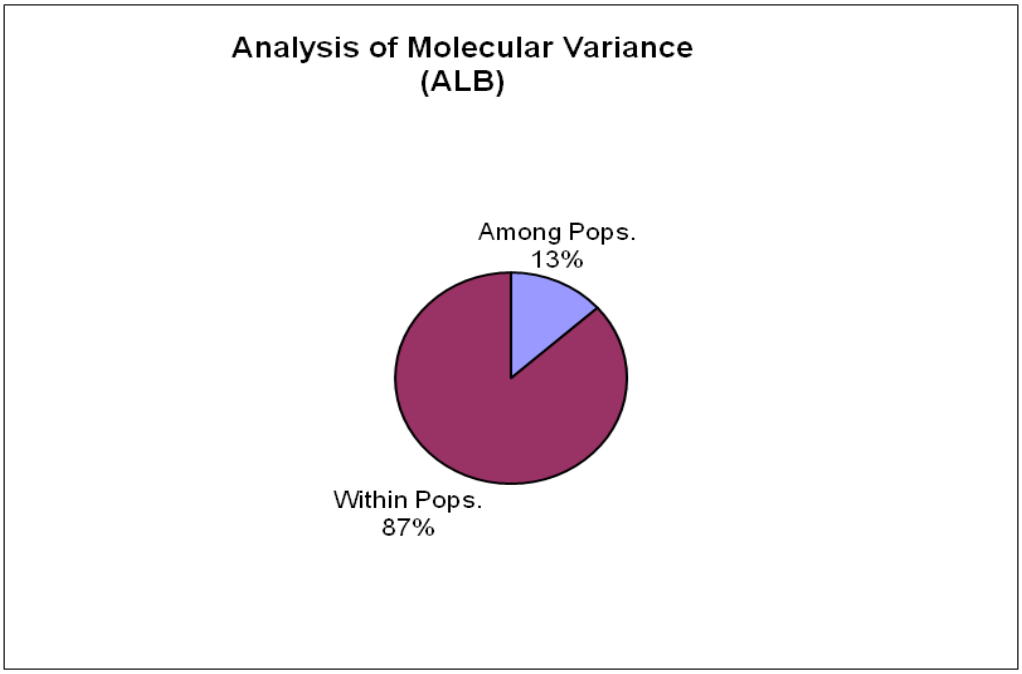

Figure 5.48 Analysis of Molecular Variance (Primer ALB-1 & ALB-2)

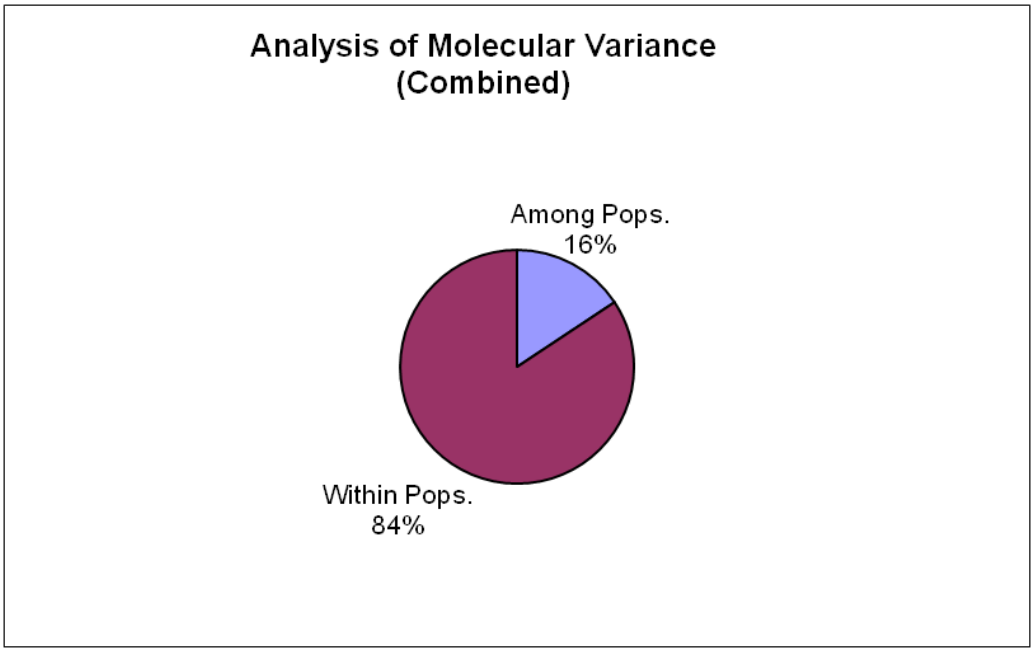

Figure 5.49 Analysis of Molecular Variance (Combined 12 Primers)

The *F*-statistics and the analysis of genetic diversity in sub-divided population have been attempted by several workers [243-245]. Long et al. [246] attempted the correlation for genetic distance between two population sub-divisions, while Cockerham [247] analysed different gene frequencies. Recently, analysis of genetic diversity among encet populations from two locations in southwestern part of Ethiopia shows higher percentage of variation between the two populations of enset as compared to within population variation [248]. Our results of high variation within the population are similar to the study on genetic divergence of two populations by Watterson [249].

CHAPTER – 6

CONCLUSIONS

CHAPTER 6.0

Conclusions

In the present investigation, the human blood samples of HIV +ve and AIDS subjects were analysed by biochemical and molecular marker analysis and $CD4^+$ cell counts. The biochemical analysis was performed for liver and kidney function tests (LFTs and KFTs), lipid profile and blood sugar. The results were subjected to non linear regression analysis.

The LFTs included determination of liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities along with total and direct bilirubin (Bil-T and Bil-D) and albumin (ALB) concentration. A comparison of enzyme activities among normal and HIV +ve subjects revealed that activities of AST, ALT and ALP enzymes were significantly higher ($p < 0.001$) in HIV +ve subjects as compared to the normal subjects in state-1 (Punjab) as well as state-2 (Rajasthan) and in all the three age groups (18-29 years, 30-39 years, 40-49 years) considered in the present investigation. The activities of these three liver enzymes further increase significantly ($p < 0.001$) in AIDS subjects as compared to the HIV +ve subjects.

Similarly, the ALB concentration in HIV +ve subjects from both the states and in all the three age groups was significantly lower ($p < 0.001$) as compared to the normal subjects. The ALB concentration was further decreased significantly ($p < 0.001$) in AIDS subjects in both the states. The Bil-T and Bil-D levels in HIV +ve subjects from both Punjab and Rajasthan were higher as compared to the normal healthy subjects. The increase in the activity of three enzymes (AST, ALT and ALP) was highly significant irrespective of distant regional locations. The lowering of concentration of ALB in HIV +ve subjects is also highly significant in the two population regions. Thus the deviations in the various liver function parameters (AST, ALT, ALP and ALB) are significantly correlated with the HIV +ve infection and its progression to AIDS condition at both the locations and three age groups considered in the present investigation.

The blood urea level in HIV subjects from the two states exhibit different significance level. The blood urea in HIV subjects from Punjab shows significantly lower concentration than their normal counterparts. The serum creatinine level also shows a lower concentration as compared to the normal subjects in Punjab state. The change in the two parameters profile may be related to the HIV infection. On the contrary, the HIV +ve subjects from Rajasthan state show practically no significant deviation in the concentration of blood urea and serum creatinine when compared with normal subjects.

When the blood urea and creatinine profiles of HIV +ve subjects from Punjab and Rajasthan were compared, the blood urea profile showed a significant difference. On the contrary, there was no significant difference in the mean creatinine concentration between the two populations. This may be attributed to the highly contrasting climate conditions leading to different osmotic balance behavior between the two populations. An age group wise analysis in higher age group (40-49 years) showed significant decrease in both blood urea and serum creatinine, possibly due to progressive renal dysfunction at higher ages.

The degree of correlation of blood urea and creatinine do not seems to be similar and consistent between two populations (Punjab and Rajasthan). Hence these two parameters may not be significant in determining HIV infection / AIDS. The same is more or less true for Bil-T and Bil-D.

The lipid profile analysis in terms of total cholesterol (TC) and triglyceride (TG) for HIV +ve subjects from both Punjab and Rajasthan states show a lower concentration of TC and higher concentration of TG. The deviation in concentration of both TC (decrease) and TG (increase) were highly significant ($p < 0.001$) in HIV +ve subjects from both the states and in all the three age groups (18-29 years, 30-39 years, 40-49 years) considered in the present investigation. The level of TC and TG differ in-significantly i.e. remain more or less same between subjects from the two states. Further, the difference is highly significant ($p < 0.001$) between HIV +ve and AIDS subjects in combined samples from both the locations.

From this observation, it may be concluded in the present investigation that the decrease in TC level and increase in TG level are strongly related with progression of HIV +ve infection to AIDS expression.

Blood glucose estimation in HIV +ve and AIDS affected subjects in the present investigation does not reveal any significant correlation with the HIV +ve infection and AIDS expression.

From the above facts, it is concluded that liver function parameters, especially enzyme activity, kidney function parameters and lipid profile are significantly correlated with HIV +ve infection leading to AIDS in the present investigation.

Our results of correlation of $CD4^+$ cell count with different biochemical parameters suggested that TG which shows a negative correlation reveals a clear and strong relationship with $CD4^+$ cell count. While, AST, ALT and ALP (showing negative correlation) and TC (showing positive correlation) reveals a clear and moderate relationship with $CD4^+$ cell count. Bil-T, Bil-D and Glu (showing negative correlation) and ALB, urea and creatinine (showing positive correlation) reveals a weak and somewhat questionable relationship with $CD4^+$ cell count. Also, a very high correlation amongst Bil-T and Bil-D (r value; 0.77) and AST – ALT (r value; 0.70) reveals a clear and strong relationship with $CD4^+$ cell count. These results can have implications in assessing HIV/AIDS, as a higher concentration of Bil-T – Bil-D and AST – ALT strongly indicates towards the decline in $CD4^+$ cell count and hence continued deterioration of immunological function which may lead to AIDS.

The $CD4^+$ cell count is a confirmatory test for HIV +ve infection and is known to be significantly correlated with HIV viral load in $CD4^+$ cell. In the present investigation, the finding that $CD4^+$ cell count is > 200 to < 500 cells / µl in HIV +ve subjects and < 200 cells / µl in fully expressed AIDS subjects is in agreement with the clinically established norms. However, $CD4^+$ cell count technique is quite costly and the facility is available at limited location.

In the present investigation, we subjected biochemical analysis results to various statistical combination and permutations to find a viable and economical technique to estimate tentative $CD4^+$ cell count. We found an answer in non linear

regression analysis (table 5.36), using regression equation $f = a * x^b * y^c$ (equations 5.1 to 5.4).

The calculation for $CD4^+$ cell count was made using four groups of biochemical parameters (AST-ALT, ALP, TC-TG, and urea-creatinine). Three of these groups (AST-ALT, ALP and TC-TG) show significant correlation with HIV infection.

The $CD4^+$ cell number obtained from the above equations; with an average ± 20% error band were within the clinical estimate limits. Therefore it may be possible to estimate $CD4^+$ cell number from two or more sets of the liver and kidney function parameters and lipid concentrations. If the estimated $CD4^+$ cell count is more or less same within ± 20% error band with all sets of parameters, HIV +ve infection may be predicted. This may considerably lower the analysis cost in resource limited settings. However our observation requires extensive evaluation to establish predictive $CD4^+$ analysis to determine HIV positive or negativity of a subject.

The molecular marker analysis leading to hierarchical cluster analysis with four marker sets together reflect more or less same results using each marker separately. The combined use of various marker sets shows the relationship patterns between various genotypes within the same population. The high variability was observed with AST-ALT primer set, possibly due to the high gene expression of AST and ALT in HIV subjects. The same is more or less true for ALP and ALB. Phylogenetic results using ALP and ALB primer sets differentiate HIV +ve subjects effectively. However it is felt that without knowing the location of various products (enzymes) on the chromosome, the results may be considerably distorted.

The results of the molecular marker analysis gave the phylogenetic clustering of HIV +ve subjects in two populations. The relationship was pronounced within the same population rather than among the two populations. The analysis of molecular variance (AMOVA) also shows the pattern with specific degree (% age) of variability within the same population as well as between the two populations. The high degree of variability among the subjects of the same population may possibly be attributed to the degree of HIV infection, thereby viral load, in a subject. However more specific

and extensive studies require microarray analysis of genomic DNA of HIV +ve subjects.

The present investigation leads to the conclusion that the liver function parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and serum albumin), lipid profile (total cholesterol and serum triglycerides) and $CD4^+$ cell count are highly and significantly correlated with each other as well as with HIV / AIDS expression. This investigation also suggests that if $CD4^+$ cell counts from both liver enzyme activity and lipid profile come with in 20% difference between each other, the subject may be considered for HIV testing. However the validity of this conclusion requires analysis of larger data from population of different regions. If the present conclusions are supported from such studies, then the process will be of low cost and can be carried out at any standard diagnostic laboratory having a simple biochemical analyser. Therefore the method will make it easy to assess HIV / AIDS status of suspects.

CHAPTER – 7

LIMITATIONS AND FUTURE SCOPE OF THE WORK

CHAPTER 7.0

Limitations and Future Scope of the Work

7.1 Limitations

The present project was designed to assess the epidemiological characteristics of HIV/AIDS affected individuals in North India. Initially we had selected five states of North India for our study. Later on due to administrative and technical problems we could be able to assess samples only from two North Indian states. Since North India comprises of around eight states, we can assume that this is one of the limitations of the current study that it does not represent the entire region of North India but rather focuses on a part of it.

A total of 319 number of blood samples (148 HIV +ve, 53 AIDS affected and 118 normal) from different individuals were accessed in the present project. This is another limitation of the project wherein the number of samples is not very large (especially AIDS affected samples) for such type of studies.

Another limitation of the study is that the HIV affected individuals were not followed up to assess their biochemical parameters over an extended period of time. Since all HIV +ve blood samples were ART naive, so the effect of ARV drugs on different biochemical parameters was also not assessed in the present study.

Because of the technical constraints and limited funds, the molecular analysis was performed with few random and some gene specific primer sequences. The number of the primers used in the study can be increased for more authentic correlations.

7.2 Future Scope

If number of states involved in the study is increased, then it can give us better representation of entire North Indian region.

Similarly a multicentre study involving high number of AIDS samples may throw more light on the epidemiological characteristics of HIV/AIDS in North Indian region.

Such kind of studies are difficult to handle by a single group of researchers, therefore several groups in the entire region must collaborate together to reveal the truer picture of the epidemiological pattern of HIV/AIDS.

The differences obtained in the biochemical test results of normal and HIV +ve subjects in our study can be used for generating marker values for predicting HIV infection.

To study the effect of HIV/AIDS on the expression pattern of different genes (bio-molecular markers), m-RNA expression analysis could be done in order to identify the effect in terms of increased or decreased expression of a particular gene.

The data generated through molecular marker analysis could be informative regarding differentiation of human population affected with HIV in India and the primers used can work as reference data for further expression pattern analysis in future studies.

The results of the biochemical analysis in the present study reveals a clear relationship, wherein either there is a strong effect of HIV/AIDS on some parameters [for example; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum albumin (ALB), total cholesterol (TC) and serum triglycerides (TG)] or very low or no effect on some other parameters [bilirubin-total (Bil-T), bilirubin-direct (Bil-D), urea, creatinine and blood glucose]. The information thus obtained can be very useful in future, large multicenter cohorts, in which only the parameters which have initially given optimum results can be studied further at large level.

REFERENCES

References

[1] Zhu T., Korber B.T., Nahmias A.J., Hooper E., Sharp P.M., Ho D.D. An African HIV-1 Sequence from 1959 and Implications for the Origin of the Epidemic. *Nature*. 1998, 391: 594-597.

[2] Centers for Disease Control and Prevention (CDC). Pneumocystis pneumonia—Los Angeles. *Morbidity and Mortality Weekly Report*. 1981, June. 30: 250-252.

[3] Sepkowitz K.A. AIDS—the first 20 years. *The New England Journal of Medicine.* 2001, 344: 1764-1772.

[4] Gupta R.M., Pradeep S., Sahni A.K., Prasad V.V., Rai A. Genomic diversity of human immunodeficiency virus type-I (HIV-1) in India. Proceedings of IAS Conference on HIV Pathogenesis and Treatment, Paris, France, 2003, Jul. [*Antiviral Therapy*. 2003, 8: abstract no. 258].

[5] Lakhashe S., Thakar M., Godbole S., Tripathy S., Paranjape R. HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis. *Journal of Biosciences*. 2008, 33: 515–525.

[6] Clavel F., Guetard D., Brun F.V., Chamaret S., Rey M.A., Ferreira M.O.S., Laurent A.G., Dauguet C., Katlama C., Rouzioux C., Klatzmann D., Champalimaud J.L., Montagnier L. Isolation of a new human retrovirus from West African patients with AIDS. *Science*. 1986, 233, 343-346.

[7] Reeves J.D., Doms R.W. Human immunodeficiency virus type 2. *Journal of General Virology*. 2002, 83: 1253-1265.

[8] Santiago M.L., Range F., Keele B.F., Li Y., Bailes E., Bibollet R.F., Fruteau C., Noe R., Peeters M., Brookfield J.F.Y., Shaw G.M., Sharp P.M., Hahn B.H. Simian Immunodeficiency Virus Infection in Free-Ranging Sooty Mangabeys (Cercocebus atys atys) from the Tai Forest, Cote d'Ivoire: Implications for the Origin of Epidemic Human Immunodeficiency Virus Type 2. *Journal of Virology*. 2005, 79: 12515–12527.

[9] Del R.C., Curran J.W. Epidemiology and prevention of acquired immunodeficiency syndrome and human immunodeficiency virus infection. In: *Principles and Practice of Infectious Diseases*. (Mandell G.L., Bennett J.E., Dolin R., eds.)7th ed. Pa: Elsevier, Philadelphia, USA. 2009, chap 1.

[10] Guss D.A. The acquired immune deficiency syndrome: an overview for the emergency physician, Part 1. *The Journal of Emergency Medicine.* 1994, 12: 375–384.

[11] Guss D.A. The acquired immune deficiency syndrome: an overview for the emergency physician, Part 2. *The Journal of Emergency Medicine.* 1994, 12: 491–497.

[12] Poropatich K., Sullivan D.J. Jr. Human immunodeficiency virus type 1 long-term non-progressors: the viral, genetic and immunological basis for disease non-progression. *Journal of General Virology*. 2011, 92: 247-268.

[13] Levy J.A. HIV pathogenesis and long-term survival. *Journal of the International AIDS Society*. 1993, 7: 1401-1410.

[14] Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. Centers for Disease Control and Prevention (CDC). 1993, [cited 2011 Mar 13]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

[15] Ruche G.L., Ramon R., Mensah A.I., Bergeron C., Diomande M., Koko S.F., Ehouman, A., Coulibaly T.K., Ekra W.C., Dabis F. Squamous intraepithelial lesions of the cervix, invasive cervical carcinoma, and immunosuppression induced by human immunodeficiency virus in Africa. *Cancer*. 1998, 82: 2401-2408.

[16] Singh A., Bairy I., Shivananda P.G. Spectrum of opportunistic infections in AIDS. *Indian Journal of Medical Sciences*. 2003, 57: 16-21.

[17] Levy J.A., Shimabukuro J. Recovery of AIDS-associated retroviruses from patients with AIDS or AIDS-related conditions and from clinically healthy individuals. *Journal of Infectious Diseases*. 1985, 152: 734-739.

[18] HIV and Its Transmission. Centers for Disease Control and Prevention (CDC). 2003, [cited 2011 Jun 24]. Available from: http://web.archive.org/web/20050204141148/http://www.cdc.gov/HIV/pubs/facts/transmission.htm

[19] Coovadia H. Antiretroviral agents—how best to protect infants from HIV and save their mothers from AIDS. *The New England Journal of Medicine.* 2004, 351: 289-292.

[20] Del R.C., Curran J.W. Epidemiology and prevention of acquired immunodeficiency syndrome and human immunodeficiency virus infection. In: *Principles and Practice of Infectious Diseases*. (Mandell G.L., Bennett J.E., Dolin R., eds.)7th ed. Pa: Elsevier, Philadelphia, USA. 2009, chap 118.

[21] Castilla J., Romero D.J., Hernando V., Marincovich B., Garcia S., Rodriguez C. Effectiveness of highly active antiretroviral therapy in reducing heterosexual transmission of HIV. *Journal of Acquired Immune Deficiency Syndromes*. 2005, 40: 96-101.

[22] Pitt J., Myer L., Wood R. Quality of life and the impact of drug toxicities in a South African community-based antiretroviral programme. *Journal of the International AIDS Society*. 2009, 12: 1-13.

[23] Dybul M., Fauci A.S., Bartlett J.G., Kaplan J.E., Pau A.K. Panel on Clinical Practices for Treatment of HIV. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Annals of Internal Medicine.* 2002, 137: 381-433.

[24] Chene G., Sterne J.A., May M., Costagliola D., Ledergerber B., Phillips A.N., Dabis F., Lundgren J., D'Arminio M.A., de Wolf F., Hogg R., Reiss P., Justice A., Leport C., Staszewski S., Gill J., Fatkenheuer G., Egger M.E. and the Antiretroviral Therapy Cohort Collaboration. Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet*. 2003, 362: 679-686.

[25] Palella F.J., Delaney K.M., Moorman A.C., Loveless M.O., Fuhrer J., Satten G.A., Aschman D.J., Holmberg S.D. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *The New England Journal of Medicine.* 1998, 338: 853-860.

[26] Mocroft A., Ledergerber B., Katlama C., Kirk O., Reiss P., d'Arminio M.A., Knysz B., Dietrich M., Phillips A.N., Lundgren J.D.; EuroSIDA study group. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet*. 2003, 362: 22-29.

[27] National AIDS Control Organisation (NACO) Press Release on HIV Estimates-2009. Ministry of Health & Family Welfare, Government of India. 2010 Dec 01. [cited 2011 Apr 15]. Available from:

http://nacoonline.org/upload/HomePage/NACO%20Press%20Release%20on%20HIV%20Estimates.pdf

[28] Aikhionbare F.O., Newman C., Womack C., Roth W., Shah K., Bond V.C. Application of random amplified polymorphic DNA PCR for genomic analysis of HIV-1-infected individuals. *Mutation Research Genomics*. 1998, 406: 25–31.

[29] Primer-BLAST. National Center for Biotechnology Information (NCBI). 2010 May. [cited 2010 May 21]. Available from: http://www.ncbi.nlm.nih.gov/tools/primer-blast/

[30] Sneath P.H.A. & Sokal R.R. UPGMA (Unweighted Pair Group Method with Arithmetic Mean. Numerical Taxonomy. W.H. Freeman and Company, San Francisco. 1973, 230-234.

[31] Kaloustian K.K.W., Sidle E.J., Selke M.H., Vedanthan R., Emmanuel K., Lillian J.B., Jebet T.V., Carroll E.A., Tierney M.W., Kimaiyo S. A model for extending antiretroviral care beyond the rural health centre. *Journal of the International AIDS Society*. 2009, 12: 1-11.

[32] Kumarasamy N., Solomon S., Sreekanth K.C., Anitha J., Cecelia S., Timothy V.F., Mayer H.K. The Changing Natural History of HIV Disease: Before and After the Introduction of Generic Antiretroviral Therapy in Southern India. *Clinical Infectious Diseases*. 2005, 41: 25–8.

[33] Easterbrook J.P., Smith M., Mullen J., Shea S., Chrystie I., Ruiter A., T.D., Geretti M.A., Zuckerman M. Impact of HIV-1 viral subtype on disease progression and response to antiretroviral therapy. *Journal of the International AIDS Society*. 2010, 13: 4, 1-9. [cited 2010 Dec 11]. Available from: http://www.biomedcentral.com/1758-2652/content/13/1/4

[34] Isaakidis P., Raguenaud M.E., Te V., Tray C.S., Akao K., Kumar V., Ngin S., Nerrienet E., Zachariah R. High survival and treatment success sustained after two and three years of first-line ART for children in Cambodia. *Journal of the International AIDS Society*. 2010, 13:11. [cited 2011 Jan 7]. Available from: http://www.biomedcentral.com/1758-2652/content/13/1/11/abstract

[35] Gomber S, Kaushik J.S., Chandra J. and Anand R. Profile of HIV infected children from Delhi and their response to antiretroviral treatment. *Indian Pediatrics*. 2011, 48: 703-707.

[36] Drapalo A.W., Jaroszewicz J., Lapinski T.W., Prokopowicz D., Rogalska M. and Parfieniuk A. Does HAART improve renal function? An association between serum cystatin C concentration, HIV viral load and HAART duration. *Antiviral Therapy*. 11: 641-645.

[37] Potter S.J., Lemey P., Achaz G., Chew C.B., Vandamme A.M., Dwyer D.E., Saksena N.K. HIV-1 compartmentalization in diverse leukocyte populations during antiretroviral therapy. *Journal of Leukocyte Biology*. 2004, 76: 562-570.

[38] Brennan T.P., Woods J.O., Sedaghat A.R., Siliciano J.D., Siliciano R.F., Wilke C.O. Analysis of Human Immunodeficiency Virus Type 1 Viremia and Provirus in Resting CD4-T Cells Reveals a Novel Source of Residual Viremia in Patients on Antiretroviral Therapy. *Journal of Virology*. 2009, 83: 8470–8481.

[39] Drain P.K., Kupka R., Mugusi F., Fawzi W.W. Micronutrients in HIV-positive persons receiving highly active antiretroviral therapy. *The American Journal of Clinical Nutrition*. 2007, 85: 333-345.

[40] Kaiser J.D., Campa A.M., Ondercin J.P., Leoung G.S., Pless R.F., Baum M.K., Micronutrient Supplementation Increases CD4 Count in HIV-Infected Individuals on Highly Active Antiretroviral Therapy: A Prospective, Double-Blinded, Placebo-Controlled Trial. *Journal of Acquired Immune Deficiency Syndromes*. 2006, 42: 523-528.

[41] Muthuraj M., Kamatchiyammal S., Usharani B., Manupriya S., Ayyappan A.R.N., Divyalakshmi K. Serum Zinc, Calcium and Albumin Levels in Pulmonary Tuberculosis Patients Co-Infected with HIV Global. *Journal of Biotechnology & Biochemistry*. 2010, 5: 27-35.

[42] Khalili H., Soudbakhsh A., Hajiabdolbaghi M., Khavidaki S.D., Poorzare A., Saeedi A.A., Sharififar R. Nutritional status and serum zinc and selenium levels in Iranian HIV infected individuals. *BMC Infectious Diseases*. 2008, 8: 165. [cited 2010 May 21] Available from: http://www.biomedcentral.com/1471-2334/8/165

[43] Miguez M.J, Lewis J.E., Bryant V.E., Rosenberg R., Burbano X., Fishman J., Asthana D., Duan R., Madhavan N., Malow R.M. Low cholesterol? Don't brag yet ...hypercholesterolemia blunt HAART effectiveness? a longitudinal study.

Journal of the International AIDS Society. 2010, 13: 25. [cited 2010 Dec 26] Available from: http://www.biomedcentral.com/1758-2652/content/13/1/25

[44] Livio A., Andrea F.S., Cynthia F., Xiangfan Y., Nigel C.J., Deborah G., Denise L., Wendy S., Emmanouil P., Ian S., Luis M.J. Metabolic and anthropometric parameters contribute to ART-mediated CD4+ T cell recovery in HIV-1-infected individuals: an observational study. *Journal of the International AIDS Society*. 2011, 14: 37. [cited 2011 Dec 27] Available from: http://www.biomedcentral.com/1758-2652/content/14/1/37

[45] Aranzabal L., Casado L.J., Moya J., Quereda C., Diz S., Moreno A., Moreno L., Antela A., Perez-Elias J.M., Dronda F., Marin A., Hernandez R.F., Moreno A., Moreno S. Influence of Liver Fibrosis on Highly Active Antiretroviral Therapy–Associated Hepatotoxicity in Patients with HIV and Hepatitis C Virus Coinfection. *Clinical Infectious Diseases*. 2005, 40: 588-593.

[46] Nishijima T., Komatsu H., Gatanaga H., Aoki1 T., Watanabe K., Kinai E., Honda H., Tanuma J., Yazaki H., Tsukada K., Honda M., Teruya K., Kikuchi Y., Oka S. Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients PLoS ONE 6(7): e22661. *doi:10.1371/journal.pone.0022661*.2011 July 25 [cited 2011 Aug 26] Available from: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0022661

[47] Kumarasamy N., Vallabhaneni S., Flanigan T.P., Mayer K.H., Solomon S. Clinical profile of HIV in India. *Indian Journal of Medical Research*. 2005, 121: 377-394.

[48] Sharma S.K., Kadhiravan T., Banga A., Goyal T., Bhatia I., Saha P.K. Spectrum of clinical disease in a series of 135 hospitalised HIV-infected patients from north India. *BMC Infectious Diseases*. 2004, 4: 52 doi:10.1186/1471-2334-4-52. 2004 Nov 22 [cited 2010 Sept 8] Available from: http://www.biomedcentral.com/1471-2334/4/52

[49] Jones J.L., Hanson D.L., Dworkin M.S., Alderton D.L., Fleming P.L., Kaplan J.E., Ward J. Surveillance for AIDS-defining opportunistic illnesses, 1992-

1997. *Morbidity and Mortality Weekly Report, CDC Surveillance Summaries*. 1999, 48: 1-22.

[50] Ramirez L.P., Barnabe C., Sartori A.M., Ferreira M.S., Tolezano J.E., Nunes E.V., Burgarelli M.K., Silva A.C., Shikanai-Yasuda M.A., Lima J.N., Da-Cruz A.M., Oliveira O.C., Guilherme C., Bastrenta B., Tibayrenc M. Clinical Analysis and Parasite Genetic Diversity In Human Immunodeficiency Virus/Chagas' Disease Coinfections In Brazil. *The American Journal of Tropical Medicine and Hygiene*. 1999, 61: 198-206.

[51] Adewole O.O., Anteyi E., Ajuwon Z., Wada I., Elegba F., Ahmed P., Betiku Y., Okpe A., Eze S., Ogbeche T., Erhabor G.E. Hepatitis B and C virus co-infection in Nigerian patients with HIV infection. *The Journal of Infection in Developing Countries*. 2009, 3: 369-375.

[52] Adler M.W. ABC of AIDS: Development of the epidemic. *British Medical Journal*. 2001, 322: 1226-1229.

[53] Lepri A.C., Sabin C.A., Phillips A.N. Investigating temporal changes in the rate of HIV progression: challenges and limitations. *Journal of the International AIDS Society*. 1997, 11: 1647-1649.

[54] Carre N., Prins M., Meyer L., Brettle R.P., Robertson J.R., McArdle H., Goldberg D.J., Zangerle R., Coutinho R.A., van den Hoek A. Has the rate of progression to AIDS changed in recent years? *Journal of the International AIDS Society*. 1997, 11: 1611-1618.

[55] Enger C., Graham N., Peng Y., Chmiel J.S., Kingsley L.A., Detels R., Munoz A. Survival from early, intermediate, and late stages of HIV infection. *The Journal of the American Medical Association*. 1996, 275: 1329-1334.

[56] Munoz A., Xu J. Models for the incubation of AIDS and variations according to age and period. *Statistics in Medicine*. 1996, 15: 2459-2473.

[57] Cohen M.S., Hellmann N., Levy J.A., DeCock K., Lange J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *The Journal of Clinical Investigation*. 2008, 118: 1244-1254.

[58] Viviana S., David D.H., Quarraisha A.K. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet*. 2006, 368: 489-504.

[59] Wang L. Overview of the HIV/AIDS epidemic, scientific research and government responses in China. *Journal of the International AIDS Society*. 2007, 21:S3-S7.

[60] Karim S.S.A., Quarraisha A.K., Gouws E., Baxter C. Global Epidemiology of HIV-AIDS. *Infectious Disease Clinics of North America*. 2007, 21: 1-17.

[61] Schacker T., Collier A.C., Hughes J., Shea T., Corey L. Clinical and Epidemiologic Features of Primary HIV Infection. *Annals of Internal Medicine*. 1996, 125: 257-264.

[62] Pedersen C, Lindhardt B.O., Jensen B.L., Lauritzen E., Gerstoft J., Dickmeiss E., Gaub J., Scheibel E., Karlsmark T. Clinical course of primary HIV infection: consequences for subsequent course of infection. *British Medical Journal*. 1989, 299: 154-157.

[63] Phillips A.N., Elford J., Sabin C., Bofill M., Janossy G., Lee C.A. Immunodeficiency and the Risk of Death in HIV Infection. *The Journal of the American Medical Association*. 1992, 268: 2662-2666.

[64] Pereyra F., Addo M.M., Kaufmann D.E., Liu Y., Miura T., Rathod A., Baker B., Trocha A., Rosenberg R., Mackey E., Ueda P., Lu Z., Cohen D., Wrin T., Petropoulos C.J., Rosenberg E.S., Walker B.D. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. *The Journal of Infectious Diseases*. 2008, 197: 563-571.

[65] Schacker T.W., Hughes J.P., Shea T., Coombs R.W., Corey L. Biological and Virologic Characteristics of Primary HIV Infection. *Annals of Internal Medicine*. 1998, 128: 613-620.

[66] Lakhashe S., Thakar M., Godbole S., Tripathy S., Paranjape R. HIV infection in India: Epidemiology, molecular epidemiology and pathogenesis. *Journal of Biosciences*. 2008, 33: 515-525.

[67] Gupta R.M., Seth P., Prasad V.V.S.P., Sahni A.K., Jena J. Detection of Human Immunodeficiency Virus Type 1(HIV-1) A/AE Circulating Recombinant Form (CRF) in India: Possible Implications. *Medical Journal Armed Forces India*. 2006, 62: 316-320.

[68] Sahni A.K., Prasad V.V., Seth P. Genomic diversity of human immunodeficiency virus type-1 in India. *International Journal of STD & AIDS*. 2002, 13:115-118.

[69] Neogi U., Sood V., Banerjee S., Ghosh N., Verma S., Samrat S., Sharma Y., Saxena A., Husain S., Ramachandran V.G., Das S., Sreedhar K.V., Goel N., Wanchu A., Banerjea A.C. Global HIV-1 molecular epidemiology with special reference to genetic analysis of HIV-1 subtypes circulating in North India: Functional and pathogenic implications of genetic variation. *Indian Journal of Experimental Biology*. 2009, 47 : 424-431.

[70] Kulkarni S.S., Lapedes A., Tang H., Gnanakaran S., Daniels M.G., Zhang M., Bhattacharya T., Li M., Polonis V.R., McCutchan F.E., Morris L., Ellenberger D., Butera S.T., Bollinger R.C., Korber B.T., Paranjape R.S., Montefiori D.C. Highly complex neutralization determinants on a monophyletic lineage of newly transmitted subtype C HIV-1 Env clones from India. *Virology*. 2009, 385: 505-520.

[71] Banerjea A.C., Sood V., Rathore A., Husain S., Khan S., Patra S., Shankar V., Kumar H., Rani N., Bano A.S., Neogi U., Ramachandran V.G. Host genes that affect progression of AIDS/HIV in India and novel gene therapeutic approaches against HIV. *Indian Journal of Biochemistry & Biophysics*. 2008, 45: 141-148.

[72] Sobti R.C., Berhane N., Mahedi S.A., Kler R., Hosseini S.A., Kuttiat V., Wanchu A. Polymorphisms of IL-6 174 G/C, IL-10 -592 C/A and risk of HIV/AIDS among North Indian population. *Molecular and Cellular Biochemistry*. 2010, 337: 145-52.

[73] Lole K.S., Bollinger R.C., Paranjape R.S., Gadkari D., Kulkarni S.S., Novak N.G., Ingersoll R., Sheppard H.W., Ray S.C. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *Journal of Virology*. 1999, 73: 152-160.

[74] Khan I.F., Vajpayee M., Prasad V.V., Seth P. Genetic diversity of HIV type 1 subtype C env gene sequences from India. *AIDS research and Human Retroviruses*. 2007, 23: 934-940.

[75] Sahni A.K., Prasad V.V., Seth P. Genomic diversity of human immunodeficiency virus type-1 in India. *International Journal of STD & AIDS*. 2002, 13: 115-118.

[76] Gurjar S.R., Mangaiarkarasi A., Ravi V., Ranga U., Desai A. Molecular Characterization of a Full-Length Genome of a HIV-2 Isolate From India. *Journal of Acquired Immune Deficiency Syndromes*. 2009, 52: 329-335.

[77] Chatterjee K. Host genetic factors in susceptibility to HIV-1 infection and progression to AIDS. *Journal of Genetics*. 2010, 89: 109-116.

[78] Papathanasopoulos M.A., Hunt G.M., Tiemessen C.T. Evolution and diversity of HIV-1 in Africa--a review. *Virus Genes*. 2003, 26: 151-163.

[79] Ari K.K., Vanham G., Arts E.J. Is HIV-1 evolving to a less virulent form in humans? *Nature Reviews Microbiology*. 2007, 5: 141-151.

[80] Sharp P.M., Hahn B.H. AIDS: Prehistory of HIV-1. *Nature*. 2008, 455: 605-606.

[81] Korber B., Gaschen B., Yusim K., Thakallapally R., Kesmir C., Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. *British Medical Bulletin*. 2001, 58: 19-42.

[82] Wilkinson E., Engelbrecht S. Molecular characterization of non-subtype C and recombinant HIV-1 viruses from Cape Town, South Africa. *Infection, Genetics and Evolution*. 2009, 9: 840-846.

[83] Grez M., Dietrich U., Balfe P., Briesen H.V., Maniar J.K., Mahambre G., Delwart E., Mullins J.I., Waigmann H.R. Genetic Analysis of Human Immunodeficiency Virus Type 1 and 2 (HIV-1 And HIV-2) Mixed Infections in India Reveals a Recent Spread of HIV-1 and HIV-2 From a Single Ancestor for each of these Viruses. *Journal of Virology*. 1994, 68: 2161-2168.

[84] Buell E.S., Salminen M.O., McCutchan F.E. Sequencing Primers for HIV-1. The Henry M. Jackson Foundation Research Laboratory and Division of Retrovirology, Walter Reed. Army Institute of Research, Rockville Md. 20850 III-15, 1995, Nov.

[85] Guimaraes M.L., Moreira A.S., Morgado M.G. Polymorphism of the Human Immunodeficiency Virus Type 1 in Brazil: Genetic Characterization of the nef

Gene and Implications for Vaccine Design. *Memorias do Instituto Oswaldo Cruz*. 2002, 97: 523-526.

[86] Paladin F.J.E., Monzon O.T., Tsuchie H., Aplasca M.R.A., Learn G.H. Jr., Kurimura T. Genetic subtypes of HIV-1 in the Philippines. *Journal of the International AIDS Society*. 1998, 12: 291-300.

[87] Robertson D.L., Gao F., Hahn B.H., Sharp P.M. Intersubtype Recombinant HIV-1 Sequences. 1997, Dec., III-25-30. Edited by: Korber B., Hahn B., Foley B., Mellors J.W., Leitner T., Myers G., McCutchan F. and Kuiken C.L. Published by: Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM.

[88] Dawood M.R., Allan R., Fowke K., Embree J., Hammond G.W. Development of Oligonucleotide Primers and Probes against Structural and Regulatory Genes of Human Immunodeficiency Virus Type 1 (HIV-1) and Their Use for Amplification of HIV-1 Provirus by Using Polymerase Chain Reaction. *Journal of Clinical Microbiology*. 1992, 30: 2279-2283.

[89] Hutchinson J.F. The Biology and Evolution of HIV. *Annual Review of Anthropology*. 2001, 30: 85-108.

[90] Zhang L., Chen Z., Cao Y., Yu J., Li G., Yu W., Yin N., Mei S., Li L., Balfe P., He T., Ba L., Zhang F., Lin H.H., Yuen M.F., Lai C.L., Ho D.D. Molecular Characterization of Human Immunodeficiency Virus Type 1 and Hepatitis C Virus in Paid Blood Donors and Injection Drug Users in China. *Journal of Virology*. 2004, 78: 13591–13599.

[91] Karsdal M.A., Kasper D. Biochemical Markers as Tools to Increase Efficiency in Drug Development. *Nordic Bioscience. Herlev AAD, and IDS Nordic, Herlev, European Musculo Skeletal Review*. 2008, 17-21.

[92] Mata-Marin J.A., Martinez, J.G., Chavarria B.H.G., Allen J.L.F., Anduiza C.I.A., Mejia A.A. Correlation between HIV viral load and aminotransferases as liver damage markers in HIV infected naive patients: a concordance cross-sectional Study. *Virology Journal*. 2009, 6: 181. doi:10.1186/1743-422X-6-181. [cited 2010 Sept 8] Available from: http://www.virologyj.com/content/pdf/1743-422X-6-181.pdf

[93] Ejilemele A.A., Nwauche C.A., Ejele O.A. Pattern of abnormal liver enzymes in HIV patients presenting at a Nigerian Tertiary Hospital. *The Nigerian Postgraduate Medical Journal*. 2007,14: 306-309.

[94] Feldmana J.G., Burnsb D.N., Gangec S.J., Bacchettid P., Cohene M., Anastosf K., Nowickig M., Delapenah R., Miottii P. Serum albumin as a predictor of survival in HIV-infected women in the Women's Interagency HIV Study. *Journal of the International AIDS Society*. 2000, 14: 863-870.

[95] Ebuehi O.A.T., Balogun M., Audu R.A, Idigbe O.E. Blood chemistry and platelet serotonin uptake as alternative method of tracking HIV/AIDS. *African Journal of Clinical and Experimental Microbiology*. 2004, 5: 155-159.

[96] Emejulu A.A., Ujowundu C.O., Igwe C.U., Ouwuliri V.A. Hepatotoxicity of Antiretroviral Drugs in HIV Seropositive Nigerian Patients. *Australian Journal of Basic and Applied Sciences*. 2010, 4: 4275-4278.

[97] Lucien K.F.H., Clement A.N.J., Fon N.P., Weledji P., Ndikvu C.P. The Effects of Antiretroviral Treatment on Liver Function Enzymes Among HIV Infected Out Patients Attending the Central Hospital of Yaounde, Cameroon. *African Journal of Clinical and Experimental Microbiology*. 2010, 11: 174-178.

[98] Ofotokun I., Smithson S.E., Lu C., Easley K.A., Lennox J. Liver Enzymes Elevation and Immune Reconstitution among Treatment-Naive HIV-Infected Patients Instituting Antiretroviral Therapy. The *American Journal of the Medical Sciences.* 2007, 334: 334-341.

[99] Price J.C., Thio C.L. Liver Disease in the HIV–Infected Individual. *Clinical Gastroenterology and Hepatology*. 2010, 8: 1002-1012.

[100] Fokunang C.N., Banin A.N., Kouanfack C,. Ngogang J.Y. Evaluation of hepatotoxicity and nephrotoxicity in HIV patients on highly active anti-retroviral therapy. *Journal of AIDS and HIV Research*. 2010, 2: 48-57.

[101] Palanisamy P., Govindaswamy B., Ganesan S., Ayyaswamy D. Changes in CD 4+ cell count, lipid profile and liver enzymes in HIV infection and AIDS patients. *Journal of Applied Biomedicine*. 2008, 6: 139–145.

[102] Joshi D., Grady J.O., Dieterich D., Gazzard B., Agarwal K. Increasing burden of liver disease in patients with HIV infection. *Lancet*. 2011, 377: 1198-1209.

[103] Puoti M., Torti C., Ripamonti D., Castelli F., Zaltron S., Zanini B., Spinetti A., Putzolu V., Casari S., Tomasoni L., Roldan E.Q., Favret M., Berchich L., Grigolato P., Callea F., Carosi G. (for the HIV-HCV Co-Infection Study Group). Severe Hepatotoxicity during Combination Antiretroviral Treatment: Incidence, Liver Histology, and Outcome. *Journal of Acquired Immune Deficiency Syndromes*. 2003, 32: 259-267.

[104] Yimer G., Aderaye G., Wondwossen A., Eyasu M., Eleni A., Lindquist L., Yamuah L., Feleke B., Aseffa A. Anti-Tuberculosis Therapy-Induced Hepatotoxicity among Ethiopian HIV-Positive and Negative Patients. PLoS ONE 3(3): e1809. doi:10.1371/journal.pone.0001809. 2008 Mar 19 [cited 2010 Dec 26] Available from: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0001809

[105] Becker S. Liver Toxicity in Epidemiological Cohorts. *Clinical Infectious Diseases*. 2004, 38: S49-S55.

[106] Oguntibeju O.O., Banjoko O. A study on the activities of liver enzymes in HIV/AIDS patients. *Journal of Medical Sciences*. 2003, 3: 106–109.

[107] Thapa B.R., Walia A. Liver Function Tests and their Interpretation. *Indian Journal of Pediatrics*. 2007, 74: 663-671.

[108] Portea R.J., Lismana T., Tripodic A., Caldwelld S.H., Trottere J.F., Coagulation in Liver Disease Study Group. The International Normalized Ratio (INR) in the MELD Score: Problems and Solutions. *American Journal of Transplantation*. 2010, 10: 1349-1353.

[109] Emejulu A.A., Onwuliri V.A., Ojiako O.A. Electrolyte Abnormalities and Renal Impairment in Asymptomatic HIV-infected Patients in Owerri, South Eastern Nigeria. *Australian Journal of Basic and Applied Sciences*. 2011, 5: 257-260.

[110] Esezobor C.I., Iroha E., Oladipo O., Onifade E., Soriyan O.O., Akinsulie A.O., Temiye E.O., Ezeaka C. Kidney function of HIV-infected children in Lagos, Nigeria: using Filler's serum cystatin C-based formula. *Journal of the International AIDS Society*. 2010, 13:17. [cited 2010 Dec 26] Available from: http://www.biomedcentral.com/content/pdf/1758-2652-13-17.pdf

[111] Emem C.P., Arogundade F., Sanusi A., Adelusola K., Wokoma F., Akinsola A. Renal disease in HIV-seropositive patients in Nigeria: an assessment of prevalence, clinical features and risk factors. *Nephrology Dialysis Transplantation*. 2008, 23: 741–746.

[112] Brook M.G., Miller R.F. HIV associated nephropathy: a treatable condition. *Sexually Transmitted Infections*. 2001, 77: 97-100.

[113] Kamga H.L.F., Assob J.C.N., Njunda A.L., Fon P.N., Nsagha D.S., Atanga M.B.S., Weledji P., Puinta D.P., Achidi E.A. The kidney function trends in human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients at the Nylon District Hospital, Douala, Cameroon. *Journal of AIDS and HIV Research*. 2011, 3: 30-37.

[114] Afhami S., Rasoulinejad M., Razeghi E., Shahriari S., Esmailpour N. Renal Disorders in HIV-Infected Patients. *Archives of Iranian Medicine*. 2007, 10: 335-338.

[115] Winston J.A., Klotman M.E., Klotman P.E.HIV-Associated Nephropathy is a Late, Not Early, Manifestation of HIV-1 Infection. *Kidney International*. 1999, 55: 1036-1040.

[116] Szczech L.A., Hoover D.R., Feldman J.G., Cohen M.H., Gange S.J., Gooze L. , Rubin N.R., Young M.A., Cai X., Shi Q., Gao W., Anastos K. Association between Renal Disease and Outcomes among HIV-Infected Women Receiving or Not Receiving Antiretroviral Therapy. *Clinical Infectious Diseases*. 2004, 39: 1199-1206.

[117] Szczech L.A., Gange S.J., Horst C.V.D., Bartlett J.A., Young M.A., Cohen M.H., Anastos K., Klassen P.S., Svetkey L.P. Predictors of Proteinuria and Renal Failure among Women with HIV Infection. *Kidney International*. 2002, 61: 195-202.

[118] Cavalcante M.A.G.D.M., Coelho S.N., Lacerda H.R. Prevalence of Persistent Proteinuria in Stable HIV/AIDS Patients and Its Association with HIV Nephropathy. *The Brazilian Journal of Infectious Diseases*. 2007, 11: 456-461.

[119] Daugas E., Rougier J.P., Hill G. HAART-Related Nephropathies In HIV-Infected Patients. *Kidney International.* 2005, 67: 393-403.

[120] Posta F.A., Holt S.G. Recent developments in HIV and the kidney. *Current Opinion in Infectious Diseases*. 2009, 22: 43-48.

[121] Janakiraman H., Abraham G., Matthew M., Kuruvilla S., Panikar V., Solomon S., Kumaraswamy, Seshan S.V., Lesley N. Correlation of CD4 Counts with Renal Disease in HIV Positive Patients. *Saudi Journal of Kidney Diseases and Transplantation*. 2008, 19: 603-607.

[122] Duggan P.F.. Activities of Enzymes in Plasma Should be Measured at 37°C. *Clinical Chemistry*. 1979, 25: 348-352.

[123] Oh J., Hegele R.A. HIV-associated dyslipidaemia: pathogenesis and treatment. *The Lancet Infectious Diseases*. 2007, 7: 787-796.

[124] Kumar A., Sathian B. Assessment of lipid profile in patients with human immunodeficiency virus (HIV/AIDS) without antiretroviral therapy. *Asian Pacific Journal of Tropical Disease*. 2011, 1: 24-27. 2011 Oct 19 [cited 2011 Dec 22] Available from: http://apjtcm.com/zz/2011apr/6.pdf

[125] Iffen T.S., Efobi H., Usoro C.A.O., Udonwa N.E. Lipid Profile of HIV-Positive Patients Attending University of Calabar Teaching Hospital, Calabar, Nigeria. *World Journal of Medical Sciences* 2010, 5: 89-93.

[126] Obirikorang C., Yeboah F.A., Quaye L. Serum Lipid Profiling in Highly Active Antiretroviral Therapy-naive HIV Positive Patients in Ghana; Any Potential Risk? Webmed Central, *Infectious Diseases*. 2010, 1(10):WMC00987. 2010 Oct 13 [cited 2010 Dec 12] Available from: http://www.webmedcentral.com/wmcpdf/Article_WMC00987.pdf

[127] Khiangte L., Vidyabat R.K., Singh M.K., Bilasini D., Singh R.T., Singh W., Gyaneshwar A. Study of Serum Lipid Profile in Human Immunodeficiency Virus (HIV) Infected Patients. *Journal, Indian Academy of Clinical Medicine*. 2007, 8: 307-311.

[128] El-Sadr W.M., Mullin C.M., Carr A., Gibert C., Rappoport C., Visnegarwala F., Grunfeld C., Raghavan S.S. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. *HIV Medicine*. 2005, 6: 114-121.

[129] Sekhar R.V., Jahoor F., Pownall H.J., Rehman K., Gaubatz J., Iyer D., Balasubramanyam A. Severely dysregulated disposal of postprandial triacylglycerols exacerbates hypertriacylglycerolemia in HIV lipodystrophy syndrome. *The American Journal of Clinical Nutrition*. 2005, 81: 1405-1410.

[130] Adewole O.O., Eze S., Betiku Ye., Anteyi E., Wada I., Ajuwon Z., Erhabor G., Lipid profile in HIV/AIDS patients in Nigeria. *African Health Sciences*. 2010, 10: 144-149.

[131] Reeds D.N., Yarasheski K.E., Fontana L., Cade W.T., Laciny E., DeMoss A., Patterson B.W., Powderly W.G., Klein S. Alterations in liver, muscle, and adipose tissue insulin sensitivity in men with HIV infection and dyslipidemia. *American Journal of Physiology-Endocrinology and Metabolism*. 2006, 290: E47-E53.

[132] Wijk V.J.P., Cabezas M.C., de Koning E.J., Rabelink T.J., Geest V.R, Hoepelman I.M. In vivo evidence of impaired peripheral fatty acid trapping in patients with human immunodeficiency virus-associated lipodystrophy. *The Journal of Clinical Endocrinology and Metabolism*. 2005, 90: 3575-3582.

[133] Riddler S.A., Smit E., Cole S.R., Li R., Chmiel J.S., Dobs A., Palella F., Visscher B., Evans R., Kingsley L.A. Impact of HIV Infection and HAART on Serum Lipids in Men. *The Journal of the American Medical Association*. 2003, 289: 2978-2982.

[134] Mellors J.W., Munoz A., Giorgi J.V., Margolick J.B., Tassoni C.J., Gupta P., Kingsley L.A., Todd J.A., Saah A.J., Detels R., Phair J.P., Rinaldo C.R. Jr. Plasma Viral Load and CD4 Lymphocytes as Prognostic Markers of HIV-1 Infection. *Annals of Internal Medicine.* 1997, 126: 946-954.

[135] Brumme Z., Wang B., Nair K., Brumme C., de Pierres C., Reddy S., Julg B., Moodley E., Thobakgale C., Lu Z., Van der Stok M., Bishop K., Mncube Z., Chonco F., Yuki Y., Frahm N., Brander C., Carrington M., Freedberg K., Kiepiela P., Goulder P., Walker B., Ndung'u T., Losina E. Impact of Select Immunologic and Virologic Biomarkers on CD4 Cell Count Decrease in Patients with Chronic HIV-1 Subtype C Infection: Results from Sinikithemba

Cohort, Durban, South Africa. *Clinical Infectious Diseases*. 2009, 49: 956-964.

[136] Peto T. Surrogate markers in HIV disease. *Journal of Antimicrobial Chemotherapy*. 1996, 37: 161-170.

[137] Paintsil E., Ghebremichael M., Romano S., Andiman W.A. Absolute CD4+ T-Lymphocyte Count as a Surrogate Marker of Pediatric HIV Disease Progression. *The Pediatric Infectious Disease Journal*. 2008, 27: 629-635.

[138] Giorgi J.V., Lyles R.H., Matud J.L., Yamashita T.E., Mellors J.W., Hultin L.E., Jamieson B.D., Margolick J.B., Rinaldo C.R. Jr., Phair J.P., Detels R., Multicenter AIDS Cohort Study. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes*. 2002, 29: 346-55.

[139] Piwowar E.M., Tugume S.B., Grant R.M., Lutalo T., Pattishall K., Katongole-Mbidde E. Beta-2 Microglobulin Values among Human Immunodeficiency Virus (HIV)-Negative, HIV-Positive Asymptomatic, and HIV-Positive Symptomatic Ugandans. *Clinical and Diagnostic Laboratory Immunology*. 1995, 2: 236-237.

[140] Katzenstein T.L. Molecular biological assessment methods and understanding the course of the HIV infection. *Acta Pathologica, Microbiologica et Immunologica Scandinavica Suppl.* 2003, 111: 1-37.

[141] Tang Y.W., Procop G.W., Persing D.H. Molecular diagnostics of infectious diseases. *Clinical Chemistry*. 1997, 43: 2021-2038.

[142] Excoffier L., Smouse P.E., Quattro J.M. Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics*. 1992, 131: 479-491.

[143] Saxena K.R., Choudhry V., Nath I., Das S.N., Paranjape S.R., Babu G., Ramlingam S., Mohanty D., Vohra H., Thomas M., Saxena B.Q., Ganguly N. K. Normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians. *Current Science*. 2004, 86: 969-975.

[144] Ray K., Gupta M.S., Bala M., Muralidhar S., Kumar J. CD4/CD8 lymphocyte counts in healthy, HIV-positive individuals & AIDS patients. *Indian Journal of Medical Research*. 2006, 124: 319-330.

[145] Uppal S.S., Verma S., Dhot P.S. Normal Values of CD4 and CD8 Lymphocyte Subsets in Healthy Indian Adults and the Effects of Sex, Age, Ethnicity, and Smoking. *Cytometry Part B Clinical Cytometry*. 2003, 52: 32-36.

[146] Oladepo D.K., Idigbe E.O., Audu R.A., Inyang U.S., Imade G.E., Philip A.O., Okafor G.O., Olaleye D., Mohammed S.B., Odunukwe N.N., Harry T.O., Ekpa M.E., Idoko J., Musa A.Z., Adedeji A., Nasidi A., Ya'aba Y., Ibrahim K. Establishment of Reference Values of CD4 and CD8 Lymphocyte Subsets in Healthy Nigerian Adults. *Clinical and Vaccine Immunology*. 2009, 16:1374-1377.

[147] Alemnji G., Mbuagbaw J., Afetane G., Nkam M. Associations Between CD4 Cell Counts and Clinical Presentations Among HIV/AIDS Patients in Cameroon. *Journal of Medical Sciences*. 2006, 6: 843-847.

[148] McGovern B.H., Golan Y., Lopez M., Pratt D., Lawton A., Moore G., Epstein M., Knox T.A. The Impact of Cirrhosis on CD4+ T Cell Counts in HIV-Seronegative Patients. *Clinical Infectious Diseases*. 2007, 44: 431-437.

[149] Ajayi A.O., Ajayi E.A., Fasakin K.A. CD4 T-Lymphocytes cell counts in adults with human immunodeficiency virus infection at the medical department of a tertiary health institution in Nigeria. *Annals of African Medicine*. 2009, 8: 257-260.

[150] Taylor J.M., Sy J.P., Visscher B., Giorgi J.V. CD4+ T-cell number at the time of acquired immunodeficiency syndrome. *American Journal of Epidemiology*. 1995, 141: 645-651.

[151] Yarchoan R., Venzon D.J., Pluda J.M., Lietzau J., Wyvill K.M., Tsiatis A.A., Steinberg S.M., Broder S. CD4 Count and the Risk for Death in Patients Infected with HIV Receiving Antiretroviral Therapy. *Annals of Internal Medicine*. 1991, 115: 184-189.

[152] Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 1990, 18: 6531-6535.

[153] Hammond J.B.W., Spanswick G.S. A Demonstration of Genomic DNA Profiling by RAPD Analysis. *Biochemical Education*. 1997, 25: 109-111.

[154] Bardakci F. Random Amplified Polymorphic DNA (RAPD) Markers. *Turkish Journal of Biology*. 2001, 25: 185-196.

[155] Ramalivhana J.N., Obi C.L., Samie A., Labuschagne C., Weldhagen G.F. Random Amplified Polymorphic DNA Typing of Clinical and Environmental *Aeromonas hydrophila* Strains from Limpopo Province, South Africa. *Journal of Health, Population and Nutrition*. 2010, 28: 1-6.

[156] Costa C.R., Silva M.R.R., Souza L.K.H., El Assal F.E., Ataide F.S., Paula C.R. RAPD Profile among *Candida albicans* isolates by using different primers. *Revista de Patologia Tropical*. 2010, 39: 41-47.

[157] Nuchprayoon S., Junpee A., Poovorawan Y. Random Amplified Polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. *Filaria Journal*. 2007, 6: 6. 2007 Jul 30. [cited 2010 Sept 23] Available from http://www.filariajournal.com/content/6/1/6

[158] Sineo L., Martini R., Borghi G., Failli M. Analysis of genetic markers by random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). *Bollettino Chimico Farmaceutico*. 1993, 132: 201-202.

[159] Pinto P.M., Resende M.A., Ito C.Y.K., Tendler M. Genetic Variability Analysis among Clinical Candida spp. Isolates Using Random Amplified Polymorphic DNA. *Memorias do Instituto* Oswaldo Cruz. 2004, 99: 147-152.

[160] The detection of HIV-l proviral DNA by PCR in clotted blood specimens. Boriskin Y.A., Booth J.C., Fernando S., Carrington D., Evans M.R., Hay P., Coates A.R.M. Journal *of Virological Methods*. 1995, 52: 87-94.

[161] Mwamburi D.M., Ghosh M., Fauntleroy J., Gorbach S.L., Wanke C.A. Predicting CD4 Count Using Total Lymphocyte Count: A Sustainable Tool for Clinical Decisions During Haart Use. *The American Journal of Tropical Medicine and Hygiene*. 2005, 73: 58-62.

[162] Badri M., Lawn S.D., Wood R. Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting.

BMC Infectious Diseases. 2008, 8. doi:10.1186/1471-2334-8-89, 2008 Jul 4. [cited 2010 Sept 18] Available from: http://www.biomedcentral.com/1471-2334/8/89

[163] Mehendale S.M., Bollinger R.C., Kulkarni S.S., Stallings R.Y., Brookmeyer R.S., Kulkarni S.V., Divekar A.D., Gangakhedkar R.R., Joshi S.N., Risbud A.R., Thakar M.A., Mahajan B.A., Kale V.A., Ghate M.V., Gadkari D.A., Quinn T.C., Paranjape R.S. Rapid Disease Progression in Human Immunodeficiency Virus Type 1-Infected Seroconverters In India. *Aids Research and Human Retroviruses*. 2002, 18: 1175–1179.

[164] Ditangco R.A., Galang H.O., Sunico E.O. Virologic, Immunologic and Clinical Determinants of HIV Disease Outcome Among Filipinos. *The Philippine Journal of Microbiology and Infectious Diseases*. 2003, 32: 169-174.

[165] Smith C.J., Sabin C.A., Lampe F.C., Shah S., Tyrer M., Youle M.S., Cropley I., Johnson M.A., Phillips A.N. The relationship between CD4 cell count nadirs and the toxicity profiles of antiretroviral regimens. *Antiviral Therapy*. 2005, 10: 459-467.

[166] Foulkes A.S., Azzoni L., Li X., Johnson M.A., Smith C., Mounzer K., Montaner L.J. Prediction-Based Classification For Longitudinal Biomarkers. *The Annals of Applied Statistics*. 2010, 4: 1476-1497.

[167] Ghate V.M., Mehendale M.S., Mahajan B.A., Yadav R., Brahme R.G., Divekar D.A., Paranjape S.R. Relationship Between Clinical Conditions and CD4 Counts In HIV-Infected Persons in Pune, Maharashtra, India. *The National Medical Journal of India*. 2000, 13: 183-187.

[168] Sabin C.A., Phillips A.N. Should HIV therapy be started at a CD4 cell count above 350 cells/μl in asymptomatic HIV-1-infected patients? *Current Opinion in Infectious Diseases*. 2009, 22: 191-197.

[169] Balakrishnan P., Solomon S., Kumarasamy N., Mayer K.H. Low-cost monitoring of HIV infected individuals on highly active antiretroviral therapy (HAART) in developing countries. *Indian Journal of Medical Research*. 2005, 121: 345-355.

[170] Langford S.E., Ananworanich J., Cooper D.A. Predictors of disease progression in HIV infection: a review. *AIDS Research and Therapy*. 2007, 4: 1-14.

[171] Phillips A.N., Sabin C.A., Elford J., Bofill M., Janossy G., Lee C.A. Use of CD4 lymphocyte count to predict long term survival free of AIDS after HIV infection. *British Medical Journal*. 1994, 309: 309-313.

[172] Madec Y., Boufassa F., Porter K., Meyer L., CASCADE Collaboration. Spontaneous control of viral load and CD4 cell count progression among HIV-1 seroconverters. *Journal of the International AIDS Society*. 2005, 19: 2001-2007.

[173] Mallardi V. The origin of informed consent. *Acta Otorhinolaryngologica Italica*. 2005, 25: 312-327.

[174] Ekouevi D.K., Becquet R., Viho I., Bequet L., Horo A., Bosse C.A., Dabis F., Leroy V. Obtaining informed consent from HIV-infected pregnant women, Abidjan, Cote d'Ivoire. *Journal of the International AIDS Society*. 2004, 18: 1486-1488.

[175] Corneli A., Vaz L., Dulyx J., Omba S., Rennie S., Behets F. The role of disclosure in relation to assent to participate in HIV-related research among HIV-infected youth: a formative study. *Journal of the International AIDS Society*. 2009, 12: 1-10.

[176] Gray R.H., Sewankambo N.K., Wawer M.J., Serwadda D., Kiwanuka N., Lutalo T. Disclosure of HIV Status on Informed Consent Forms Presents an Ethical Dilemma for Protection of Human Subjects. *Journal of Acquired Immune Deficiency Syndromes*. 2006, 41: 246-248.

[177] Sastry J., Pisal H., Sutar S., Kundu N.K., Joshi A., Suryavanshi N., Bharucha K.E., Shrotri A., Phadke M.A., Bollinger R.C., Shankar A.V. Optimizing the HIV/AIDS informed consent process in India. *BMC Medicine*. 2004, 2:28 doi:10.1186/1741-7015-2-28. 2004 Aug 2. [cited 2010 Sept 28] Available from:
http://www.biomedcentral.com/1741-7015/2/28/comments

[178] Rao A.S.R.S., Thomas K., Sudhakar K., Maini P.K. HIV/AIDS Epidemic in India and Predicting the Impact of the National Response: Mathematical

Modeling and Analysis. *Mathematical Biosciences and Engineering*. 2009, 6: 779-813

[179] Sehgal S. HIV epidemic in Punjab, India: time trends over a decade. *Bulletin of the World Health Organization*. 1998, 76: 509-513.

[180] National AIDS Control Organisation (NACO). Annual report 2009-10. Department of AIDS Control, Ministry of Health & Family Welfare, Government of India. 2010 Mar. [cited 2010 Apr 25] Available from: http://www.nacoonline.org/Quick_Links/Directory_of_HIV_Data/

[181] National AIDS Control Organisation (NACO). Categorisation of Districts based on HIV Sentinel Suveillance 2004, 2005, 2006. Ministry of Health & Family Welfare, Government of India. 2007. [cited 2010 Mar 19] Available from: http://nacoonline.org/upload/NACO%20PDF/District%20Categorisation%20for%20Priority%20Attention.pdf

[182] State of Literacy. Provisional Population Totals Paper 1 of 2001 India Series 1. Chapter 7. Census of India 2001. 2002. [cited 2010 May 18] Available from: http://censusindia.gov.in/2011-prov-results/data_files/india/Final%20PPT%202011_chapter6.pdf

[183] Migration Tables. Data Highlights. Census of India 2001. 2002. [cited 2010 Apr 22] Available from: http://censusindia.gov.in/Data_Products/Data_Highlights/Data_Highlights_link/data_highlights_D1D2D3.pdf

[184] Manual on Quality Standards for HIV Testing Laboratories. National AIDS Control Organisation (NACO). Ministry of Health & Family Welfare, Government of India. 2007 March. [cited 2009 Feb 5] Available from: http://www.nacoonline.org/upload/Blood%20Saftey/Manual%20on%20Quality%20Standads%20for%20HIV%20Testing%20Laboratories.pdf

[185] IFCC Method for aspartate aminotransferase. *Journal of Clinical Chemistry & Clinical Biochemistry*. 1986, 24: 49.

[186] IFCC Method for alanine aminotransferase. *Journal of Clinical Chemistry & Clinical Biochemistry*. 1986, 24: 481-489.

[187] Bablok W., Passing H., Bender R., Schneider B. A General Regression Procedure for Method Transformation. Journal of Clinical *Chemistry & Clinical Biochemistry*. 1988, 26: 783-790.

[188] Doumas B.T., Watson W.A., Biggs H.G. Albumin standards and the measurement of serum albumin with bromcresol green. 1971. *Clinica Chimica Acta*. 1997, 258: 21-30.

[189] Jendrassik L., Grof P. Colorimetric Method of Determination of Bilirubin. *Biochem Z*. 1938, 297: 81-82.

[190] Tietz N.W. Clinical Guide to Laboratory Tests 3rd ed. Philadelphia, Pa. WB Saunders Company. 1995: 624, 622-629.

[191] Foster-Swanson A., Swartzentruber M., Roberts P., Feld R., Johnson M., Wong S., Reference Interval Studies of the Rate-Blanked Creatinine/Jaffe Method on BM/Hitachi Systems in Six U. S. Laboratories. *Clinical Chemistry*. 1994, 40: 1057. Abstract No 361.

[192] Roeschlau P., Bertnt E., Gruber W.A. Enzymatic determination of total cholesterol in serum. Journal *of Clinical Chemistry & Clinical Biochemistry*. 1974, 12: 226.

[193] Wahlefeld A.W. Triglycerides determination after enzymatic hydrolosis. In: Bergmeyer H.U. ed Methods of enzymatic analysis 2nd English ed Verlag Chemie Weinheim and Academic Press. New York and London. 1974, 4: 1831.

[194] Tietz N.W. (Hrsg). Clinical Guide to Laboratory Tests, 3. Auflage. Philadelphia. PA: WB Saunders Company: 1995: 266-273.

[195] Hirata R.D.C., Salazar L.A., Hirata M.H., Cavalli S.A., Machado M.O. Optimized Procedure for DNA Isolation from Fresh and Cryopreserved Clotted Human Blood Useful in Clinical Molecular Testing. *Clinical Chemistry*. 1998, 44: 1748-1750.

[196] Maniatis T., Fritsch E.F., Sambrook J. *Molecular Cloning - A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Plainview, New York, 1982.

[197] Johnson P.H., Grossman L.I. Electrophoresis of DNA in agarose gels. Optimizing separations of conformational isomers of double and single-stranded DNAs. *Biochemistry*. 1977, 16: 4217-4225.

[198] Sokal R., Michener C.A. Statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin*. 1958, 38: 1409-1438.

[199] Botstein D., White R.L., Skolnick M., Davis R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. 1980, 32: 314-331.

[200] Prevost A., Wilkinson M.J. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics*. 1999, 98: 107-112.

[201] Oosthuizen W., Graan A.V., Kruger A.K., Vorster H.H. Polyunsaturated fatty acid intake is adversely related to liver function in HIV-infected subjects: the THUSA study. *The American Journal of Clinical Nutrition*. 2006, 83: 1193-1198.

[202] Stabinski L., Reynolds S.J., Ocama P., Laeyendecker O., Ndyanabo A., Kiggundu V., Boaz I., Gray R.H., Wawer M., Thio C., Thomas D.L., Quinn T.C., Kirk G.D., Rakai Health Sciences Program. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. *Antiviral Therapy*. 2011, 16: 405-411.

[203] Sinicco A., Palestro G., Caramello P., Giacobbi D., Giuliani G., Paggi G., Sciandra M., Gioannini P. Acute HIV-1 infection: clinical and biological study of 12 patients. *Journal of Acquired Immune Deficiency Syndromes*. 1990, 3: 260-265.

[204] Blacker J. The impact of AIDS on adult mortality: evidence from national and regional statistics. *Journal of the International AIDS Society*. 2004, 18(supl 2): S19-S26.

[205] Lee R.D., Nieman D.C. Nutritional assessment. Madison, WI: Wm C Brown Communications, Inc, 1993.

[206] Meenan J., Mooney E., Mosquita N., Johnson A.H., Collins P., Feely J., Mulcahy F.M. The impact of HIV disease progression on serum lipoproteins. *Journal of the International AIDS Society*. 1992, 6: 1551-1552.

[207] Emem C.P., Arogundade F., Sanusi A., Adelusola K., Wokoma F., Akinsola A. Renal disease in HIV-seropositive patients in Nigeria: an assessment of

prevalence, clinical features and risk factors. *Nephrology Dialysis Transplantation*. 2008, 23: 741-746.

[208] Akpa M.R., Agomoah D., Alasia D.D. Lipid profile of healthy adult Nigerians in Port Harcourt, Nigeria. *Nigerian Journal of Medicine*. 2006, 2: 137-140.

[209] Okafor C.I., Fasanmade O.A., Oke D.A. Pattern of dyslipidaemia among Nigerians with Type 2 diabetes mellitus. *Nigerian Journal of Clinical Practice*. 2008, 11: 25-31.

[210] Buchacz K., Weidle P.J., Moore D. Changes in lipid profiles over 24 months in adults on first line highly active antiretroviral therapy in the home based care in rural Uganda. *Journal of Acquired Immune Deficiency Syndromes*. 2008, 47: 304-311.

[211] Periard D., Telenti A., Sudre P., Cheseaux J.J., Halfon P., Reymond M.J., Marcovina S.M., Glauser M.P., Nicod P., Darioli R., Mooser V. Atherogenic dyslipidemia in HIV infected individuals treated with protease inhibitors. The Swiss HIV cohort study. *Circulation*. 1999, 100: 700-705.

[212] Mondy K., Overton E.T., Grubb J., Tong S., Seyfried W., Powderly W., Yarasheski K. Metabolic Syndrome in HIV- infected patients from an urban Midwestern US outpatient population. *Clinical Infectious Diseases*. 2007, 44: 726-734.

[213] Mullamitha S.A., Pazare A.R. Study of Lipid profiles in HIV infection. *Journal of the Association of Physicians of India*. 1999, 47: 622-624.

[214] Young J., Weber R., Rickenbach M., Furrer H., Bernasconi E., Hirschel B., Tarr P.E., Vernazza P., Battegay M., Bucher H.C. Lipid Profiles for antiretroviral-naive patients starting PI-and NNRTI-based therapy in the Swiss HIV cohort study. *Antiviral Therapy*. 2005, 10: 585-591.

[215] Grunfeld C., Kotler D.P., Shigenaga J.K., Doerrler W., Tierney A., Wang J., Pierson R.N. Jr., Feingold K.R. Circulating Interferon-alpha levels and Hypertriglyceridaemia in the Acquired Immune Deficiency Syndrome. *American Journal of Medicine*. 1991, 90: 154-162.

[216] Grunfeld C., Pang M., Doerrler W., Shigenaga J.K., Jensen P., Feingold K.R. Lipids, Lipoproteins, Triglyceride Clearance and Cytokines in Human Immunodeficiency Virus Infection and the Acquired Immunodeficiency

Syndrome. *The Journal of Clinical Endocrinology and Metabolism*. 1992, 74: 1045-1052.

[217] Gadd C. Metabolic disturbances in the absence of HAART. *The International Association of Physicians in AIDS Care Monthly*. 2005, 11: 98-99. 2005 Apr 1 [cited 2010 Sept 22] Available from: http://www.thebody.com/content/art12187.html

[218] Spollett G.R. Hyperglycemia in HIV/AIDS. *Diabetes Spectrum*. 2006, 19: 163-166.

[219] Hommes M.J., Romijn J.A., Endert E., Schattenkerk J.K.E., Sauerwein H.P. Insulin sensitivity and insulin clearance in human immunodeficiency virus-infected men. *Metabolism*. 1991, 40: 651-656.

[220] Heyligenberg R., Romijn J.A., Hommes M.J., Endert E., Schattenkerk J.K.E., Sauerwein H.P. Non-insulin mediated glucose uptake in human immunodeficiency virus-infected men. *Clinical Science*. 1993, 84: 209-216.

[221] Mulligan K., Grunfeld C., Hellerstein M. K., Neese R.A., Schambelan M. Anabolic effects of recombinant human growth hormone in patients with wasting associated with human immunodeficiency syndrome. *Journal of Clinical Endocrinology and Metabolism*. 1993, 77: 956-962.

[222] Dube M.P. Disorders of glucose metabolism in patients infected with human immunodeficiency virus. *Clinical Infectious Diseases*. 2000, 31: 1467-1475.

[223] Carr A., Samaras K., Thorisdottir A., Kaufmann G.R., Chisholm D.J., Cooper D.A. Diagnosis, prediction, and natural course of HIV-1 protease inhibitor-associated lipodystrophy, hyperlidaemia and Diabetes mellitus: a cohort study. *The Lancet*. 1999, 335: 2093-2099.

[224] Howard A.A., Floris-Moore M., Arnsten J.H., Santoro N., Fleischer N., Lo Y., Schoenbaum E.E. Disorders of glucose metabolism in HIV-infected women. *Clinical Infectious Diseases*. 2005, 40: 1492-1499.

[225] Ranga U., Banerjea A., Chakrabarti S., Mitra D. HIV/AIDS research in India: Past, present and future. *Current Science*. 2010, 98: 335-345.

[226] Gandhi R.T. Cirrhosis is associated with low CD4+ T cell counts: implications for HIV-infected patients with liver disease. *Clinical Infectious Diseases*. 2007, 44: 438-440.

[227] Jaroszewicz J., Wiercinska-Drapalo A., Lapinski T.W., Prokopowicz D., Rogalska M., Parfieniuk A. Does HAART improve renal function? An association between serum cystatin C concentration, HIV viral load and HAART duration. *Antiviral Therapy*. 2006, 11: 641-645.

[228] Szczech L.A., Gupta S.K., Habash R., Guasch A., Kalayjian R., Appel R., Fields T.A., Svetkey L.P., Flanagan K.H., Klotman P.E., Winston J.A. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney International*. 2004, 66: 1145-1152.

[229] Post F.A., Wood R., Maartens G. CD4 and total lymphocyte counts as predictors of HIV disease progression. *Quarterly Journal of Medicine*. 1996, 89: 505-508.

[230] Spacek L.A., Griswold M., Quinn T.C., Moore R.D. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. *Journal of the International AIDS Society*. 2003, 17: 1311-1317.

[231] Ferris D.C., Dawood H., Magula N.P., Lalloo U.G. Application of an algorithm to predict CD4 lymphocyte count below 200 cells/mm^3 in HIV-infected patients in South Africa. *Journal of the International AIDS Society*. 2004, 18: 1481-1482.

[232] Mellors J.W., Margolick J.B., Phair J.P., Rinaldo C.R., Detels R., Jacobson L.P., Munoz A. Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 Cell count slope for progression to AIDS and death in untreated HIV-1 infection. *The Journal of the American Medical Association*. 2007, 297: 2349-2350.

[233] Abdelmigid H.M. Efficiency of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers forgenotype fingerprinting and genetic diversity studies in canola (Brassica napus). *African Journal of Biotechnology*. 2012, 11, 6409-6419.

[234] Pillen K., Binder A., Kreuzkam B., Ramsay L., Waugh R., Forster J., Leon J. Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. *Theoretical and Applied Genetics*. 2000, 101: 652-660.

[235] Hamza S., Hamida B.W, Rebai A., Harrabi M. SSR based genetic diversity assessment among Tunisian Winter Barley and relationship with morphological traits. *Euphytica.* 2004, 135: 107-118.

[236] Melchinger A.E., Utz H.F., Schon C.C. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics*. 1998, 149: 383-403.

[237] Winkelman A.M., Peterson R.G. Heritabilities, dominance variation, common environmental effects and genotype by environment interactions for weight and length in chinook salmon. *Aquaculture*. 1994, 125: 17-30.

[238] Atkin A., Pestano G., Serwadda D., Prince A.M., Pascual D., Sewankambo N., Boto W.M. Phylogenetic and serological characterization of two Ugandan HIV-1 isolates. *AIDS Research and Human Retroviruses*. 1993, 9: 351-356.

[239] McClelland M., Welsh J. DNA fingerprinting by arbitrarily primed PCR. *Genome Research*. 1994, 4: S59-S65.

[240] Williams J.G.K., Hanafey M.K., Rafalski J.A., Tingey S.V. Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology*. 1993, 218: 704-740.

[241] Pan Y.B., Burner D.M., Ehrlich K.C., Grisham M.P., Wei Q. Analysis of primer-derived, nonspecific amplification products in RAPD-PCR. *BioTechniques*. 1997, 22: 1071-1077.

[242] Mbwana J., Bolin I., Lyamuya E., Mhalu F., Lagergard T. Molecular characterization of Haemophilus ducreyi isolates from different geographical locations. Journal of Clinical Microbiology. 2006, 44: 132-137.

[243] Long J.C. The allelic correlation structure of Gainj- and Kalam-speaking people. The estimation and interpretation of Wright's F-statistics. *Genetics*. 1986, 112: 629-647.

[244] Nei M. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*. 1973, 70: 3321-3323.

[245] Nei M. F-statistics and the analysis of gene diversity in subdivided populations. *Annals of Human Genetics*. 1977, 41: 225-233.

[246] Long J.C., Smouse P.E., Wood J.W. The allelic correlation structure of Gainj- and Kalam-speaking people. The genetic distance between population subdivisions. *Genetics*. 1987, 117: 273-283.

[247] Cockerham C.C. Analyses of gene frequencies. *Genetics*. 1973, 74: 679-700.

[248] Tobiaw D.C., Bekele E. Analysis of genetic diversity among cultivated enset (*Ensete ventricosum)* populations from Essera and Kefficho, southwestern part of Ethiopia using inter simple sequence repeats (ISSRs) marker. *African Journal of Biotechnology*. 2011, 10: 15697-15709.

[249] Watterson G.A. The genetic divergence of two populations. *Theoretical Population Biology*. 1985, 27: 298-317.

APPENDICES

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MIET/IRB/PhD/001/2010-11

11-08-2010

The Institutional Research Board (IRB) in his meeting held on 10-08-2010 discussed the project entitled "**Epidemiological Study on HIV and AIDS Affected Population in North India**" [Previously "Epidemiological study of Acquired Immune Deficiency Syndrome (AIDS) in North India"], submitted by Mr. Ajay Kumar Sharma, Assistant Professor, Department of Biotechnology, MIET, Meerut.

The following members were present:

1. Lt. Gen. J. M. Garga (Director)
2. Prof. S. P. Gupta (Member)
3. Prof. A. Subrahmanyam (Member)
4. Prof. D. K. Sharma (Member)
5. Sh. S. K. Sharma (Member)

The Board unanimously approved the above said project. The Institutional Research Board has no objection for the conduct of above mentioned programme in MIET, Meerut or other centres as mentioned in the proposal. The project has to be conducted as per the research guidelines of MIET or other institutions.

MEERUT INSTITUTE OF ENGINEERING
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MEERUT-250002

(Director)

Institutional Research Board (IRB)

Meerut Institute of Engineering & Technology (MIET), Meerut

Copy to: Registrar, MIET

Mr. Ajay Kumar Sharma, MIET

miet GROUP OF INSTITUTIONS

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MIET/IEC/PhD/002/2010-11

09-09-2010

The Institutional Ethical Committee (IEC) meeting was held on 08-09-2010 to discuss the project entitled "**Epidemiological Study on HIV and AIDS Affected Population in North India**" [Previously "Epidemiological study of Acquired Immune Deficiency Syndrome (AIDS) in North India"], submitted by Mr. Ajay Kumar Sharma, Assistant Professor, Department of Biotechnology, MIET, Meerut.

The following members were present:

1. Dr. P. S. Agarwal (Chairman)
2. Dr. Manish Agarwal (Member)
3. Dr. Tanuraj Sirohi (Member)
4. Mr. Pramod Kumar Garg (Member)
5. Dr. Anjali Bharti (Member)
6. Dr. Niyati Garg (Member)
7. Mr. Lalit Agarwal (Member)
8. Mr. Sanjay Vashisht (Member Secretary)

The committee unanimously approved the above said project. The Institutional Ethical Committee has no objection for the conduct of above mentioned programme. The project has to be conducted as per the ethical guidelines of ICMR, 2006 and the concerned investigators have to submit the yearly progress report in the office of the undersigned.

(Chairman)

Institutional Ethical Committee (IEC)

Meerut Institute of Engineering & Technology (MIET), Meerut

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Mr. Ajay Kumar Sharma, MIET

List of Publications

- Sharma A.K., Sharma V.N., Kalla N.R., Sharma V. Random and Gene Specific Amplification as Tool for Phylogenetic Assessment: A Study of HIV Subjects From North India. *International Journal of Science and Technology*. 2012, 1: 384-390.
- Sharma A.K., Sharma V.N., Kalla N.R., Singh A. Effect of HIV/AIDS on Liver Function Tests in North Indian Population. *International Journal of Science and Advanced Technology*. 2012, 2: 38-44.
- Sharma A.K., Singh A., Garg N., Sirohi S., Sharma V. Intellectual Property Rights: Perspectives in HIV/AIDS Management. Presented in workshop on "*Intellectual Property Rights and Management for Engineering & Technology disciplines*" at NCCE, Israna (March 30-31, 2012).
- Sharma A.K., Sharma V.N. HIV/AIDS - related opportunistic infections among people in North India. Presented in "*National Conference on Emerging Trends in Life Sciences Research"* (ETLSR-2009) at BITS, Pilani (March 6-7, 2009).

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- Ph.D. (Cell Biology) from Panjab University (1969)
- M.Sc. (Zoology) from Rajasthan University (1963)
- B.Sc.(BCZ) from Panjab University (1961)
- Matriculation from Rajputana University (1957)

Experience :
Research : 45 years

Areas of interest : Cell Biology (Cytochemistry and Liposomes), Biotechnology (Biomonitoring Biodegradation and Bioremediation) and Bioenergy (Alternate energy sources).

Publication : Research > 70, Book 01, Lecture Notes 03

Supervision : M. Pharm. Thesis 03 :

M.Sc.-Project >70 + Thesis > 50
Ph.D. – Awarded 08 + Submitted 01 + Working 02 =11
Evaluated >25

Teaching : > 42 years to both UG and PG classes

Subjects : Cell Biology, Genetics, Biochemistry, Development Biology, Reproductive Biology, Molecular Biology, Enzymology, Industrial Microbiology, Fermentation Technology, Liposome Technology, Cell & Tissue Culture, etc.

Publications (Last five years)

- Sharma A.K., Sharma V.N., Kalla N.R., Sharma V. Random and Gene Specific Amplification as Tool for Phylogenetic Assessment: A Study of HIV Subjects From North India. *International Journal of Science and Technology*. 2012, 1: 384-390.
- Sharma A.K., Sharma V.N., Kalla N.R., Singh A. Correlation of Liver Function Tests with HIV/AIDS in North Indian Population. *International Journal of Science and Advanced Technology*. 2012, 2: 38-44.
- Agarwal S., Kulkarni G.T., Sharma V.N. A comparative study on the antioxidant activity of methanol extracts of *Terminalia paniculata* and *Madhuca longifolia*. *Free Radicals and Antioxidants*. 2011, 1: 62-68.
- Agarwal S., Kulkarni G.T., Sharma V.N. A comparative study on the antioxidant activity of methanol extracts of *Acacia nilotica* and *Berberis chitria*. *Advances in Natural and Applied Sciences*. 2010, 4: 78-84.
- Garg N., Gaurav S.S., Sharma V.N., Singhal G., Neeraj. Morphological characterization of cultivars of safed musli *Chlorophytum borivilianum*. *Journal of Scientific and Applied Research*. 2010, 1: 52-55.
- Mathuriya A.S., Sharma V.N. Bioelectricity production from paper industry waste using a microbial fuel cell by Clostridium species. *Journal of Biochemical Technology*. 2009, 1: 49-52.
- Mathuriya A.S., Sharma V.N. Bioelectricity production from various waste waters through Microbial Fuel Cell Technology. *Journal of Biochemical Technology*. 2010, 2: 133-137.
- Mathuriya A.S., Sharma V.N. Treatment of Brewery Wastewater and production of Electricity through Microbial Fuel Cell Technology. *International Journal of Biotechnology and Biochemistry*. 2010, 6: 72-80.
- Mathuriya A.S., Sharma V.N. Electricity generation from potato waste water in a microbial fuel cell. *International Journal of Agriculture, Environment and Biotechnology*. 3: 335-340.

- Mathuriya A.S., Sharma V.N. Bioelectricity production by *Saccharomyces cerevisae* from Sugar Industry waste using Microbial Fuel Cell Technology. *Journal of Pure and Applied Microbiology*. 2010, 4: 31-40.
- Mathuriya A.S., Sharma V.N. Electricity generation by Saccharomyces cerevisae and Clostridium acetobutylicum via microbial fuel cell technology: A comparative study Advances in Biological Research. 2010, 4: 217-223.
- Sharma A.K., Sharma V.N. HIV/AIDS - related opportunistic infections among people in North India. Presented in: *National Conference on Emerging Trends in Life Sciences Research (ETLSR-2009)*. BITS, Pilani. March 6-7, 2009.

Membership :

- Fellow Theosophical Society of India.
- Fellow Academy of Science (A.W.).
- Life member of Indian Science Congress.
- Life member of Society of Biosciences.
- Life member of North India Science Association.
- Life member of Andrology Society of India.
- Member I.S.T.E.

Positions Held :

Research:

-CSIR– PDF at P.U., Chandigarh (1968-69).
-CSIR – SRF at P.U. Chandigarh (1967-68)
-CSIR - JRF at P.U. Chandigarh (1965-67).
-CSIR- JRF at Jodhpur. University (1963-65).

Teaching :

- Emeritus Professor of Biotechnology at M.I.E.T., Meerut (Aug, 2012 -contd.)
- Visiting Professor of Biotechnology at M.I.E.T., Meerut (2011 – July, 2012).
- Professor of Biotechnology at M.I.E.T., Meerut (Dec. 2004–Nov.2010).
- Professor of Biotechnology at Sobhasaria Engg. College Sikar (2004).
- Professor of Biotechnology at N.C.College, Israna (2003)
- Visiting Professor Bio-sci. & Biotech. at BITS., Pilani (2002-2003)
- Professor of Bio-sci. & Biotech. at BITS, Pilani (1991-2002).
- Associat Professor of Bio-sciences at BITS, Pilani (1986 -1991).
- Assistant Professor of Bio-sciences at BITS, Pilani (1976-1986).
- Lecturer in Bio-sciences at BITS, Pilani (1969 -1976)

Administrative :

- Nucleus Member of Educational Hardware Division (Incharge Financial planning and consumables), BITS, Pilani (1976-84).
- Member of Central Instrument Lab. Advisory committee,BITS, Pilani (1978-2002).
- Group-leader (Head) of Biosciences Group (Dept.),(1991-1993) And Bioscience & Biotech. Group , BITS , Pilani (1993-1996).
- Head of Biotech. Dept. at N.C. Engg.College, Israna (2003)
- Head of Biotech. Dept. at Sobhasaria Engg. College, Sikar (2004).
- Head of Biotech. Dept. at M.I.E.T., Meerut. (2005).
- Director, Biotechnology Programme, MIET, Meerut (2006 – 2010).

Seminar/ Conf. / Workshops:

Attended : Attended many National and International gatherings.

Organised : Some of the seminars/workshop/Conf. organized in last five years as Chairman:

- XII Annual Conference of Andrological Society (2005).
- National Seminar on Apoptosis (2006).
- Workshop on Advances in Modern Biology in 2007 (2months).
- Workshop on Bioinformatics in collaboration with Mascon India.(2008).
- Workshop on Advances in Research Techniques in Reproductive Biomedicine (2009) sponsored by ICMR.

Resource Person [**Training & Workshop]:**

- Teachers training workshop at Rajasthan University Staff College Jaipur (1993-1995).
- AICTE sponsored pharmacy workshop at Nagpur Univ. (1999).
- MHRD sponsored workshop on Mol. Biology at BITS, Pilani (2000)
- A VC & Principal/Director Level Workshop on “Curriculum and Syllabus Development: Biotechnology section” convened by Directorate of Technical Education, Haryana Govt. (2003).
- Workshop on “Biotechnology Development” Convened by Technical Education Ministry, Rajasthan Govt. (2004).

Curriculum Development:

Course Development

- Development of Syllabus for M.E. (Biotech.) at BITS, Pilani (1993).
- M. Tech (Biotech) Course Developed for UPTU (2005).

- Developed M.Tech. (Bioinformatics) Syllabus at UPTU (2009).

External expert of "Board of studies" on

- Zoology at Raj. Univ., Jaipur. (1993-1995).
- Life sciences at M.D. Univ., Rohtak. (1996-1999).
- Microbiology at S.M.D. Univ., Ajmer (2003-2005).
- Biotechnology at Raj, Tech. Univ., (2004-2005).
- Food Science at Raj. Univ., Jaipur (2004-2006).
- Biotechnology at UPTU, Lucknow (2008).

Selection Committees:

External Subject Expert for selection committees at Panjab Univ., Rajasthan Univ., Bundelkhand Univ., CEERI (Pilani), UPTU affiliated Colleges, etc.

UGC Committee:

Member UGC review committee on IX & X plan for Assam Univ. (2003).

Evaluation Work:

Expert to evaluate Research and Academic progress of faculty at Various Universities.
Head Examiner for Public service commission of Bihar (1991-92) and Haryana (1998).

Laboratory Development:

- Involved in the development of Central Lab. at BITS, Pilani (1978).
- Involved in the development of Genetic Engg. Lab. BITS (1979).
- A new laboratory complex infrastructure was developed at N.C.College of Engineering, Israna (Panipat). (2003)
- An Advanced Biotechnology Lab. was developed at MIET, Meerut. (2005 -2006).

(V. N. Sharma)

Curriculum Vitae

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: +91-120-2777806 (Res.)

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EDUCATIONAL QUALIFICATIONS

Education	Institute/University	Year of Completion	Grade/ Percentage
Ph.D.	Birla Institute of Technology and Science (BITS), Pilani, Rajasthan.	Submitted Thesis (Dec., 2012)	--
Master of Engineering (Biotechnology)	Birla Institute of Technology and Science (BITS), Pilani, Rajasthan.	June, 2002	C.G.P.A. 8.19
Bachelor of Pharmacy	Maharishi Dayanand University (MDU) Rohtak, Haryana.	June, 2000	61.11 %

PROFESSIONAL EXPERIENCE (> 11 Years)

Academics & Research : 10 years
Industry : 1 year

Presently working as Assistant Professor in the Department of Biotechnology at Meerut Institute of Engineering & Technology (MIET), Meerut, since 1-02-2004.

KEY SKILLS & ABILITIES DEMONSTRATED (Past Five Years)

- **Taught** several **inter-disciplinary subjects** in the area of Genetic Engineering, Genetics & Molecular Biology, Pharmaceutical Biotechnology, and Immunology to U.G. and P.G. students.
- **Trained students** in various **practical skills** of:
  - Isolation & Purification of DNA from bacterial and blood cells
  - Partial and Complete Restriction Digestion
  - Polymerase chain reaction (PCR)
  - Bacterial Transformation and Conjugation
  - Southern and Western Blotting
  - Histochemical staining of enzymes
  - Immunological Techniques (including ELISA)
- ***Supervised 06 Projects*** of **M. Tech. &** ***10 Projects*** of **B. Tech. students** in their final semester research projects.

KEY ACHIEVEMENTS

- **Performed a key role** in preparing the ***proposal for Accreditation*** (through NBA) which finally **results in** *Accreditation of B. Tech. Biotechnology Programme* at MIET (2007, 2012).
- **Appreciated** by the Institute for **"Improving Teaching-Learning"** process (December, 2009 & March, 2011).
- **Conducted** an **"AIDS Awareness Survey"** in three different academic institutions of Meerut as part of student's research project (April, 2008).
- **Instrumental in forming MIET Biotechnology Society (Greater than 700 members)** in November, 2006 and **launching** its first publication—**"Biotaction"** magazine in December, 2007.
- **Adjudged** as one of the **Top Performer in Teaching (out of total 150 faculties)** on the basis of Student's Result and their feedback (October, 2006).

PUBLICATIONS

- Sharma A.K., Sharma V.N., Kalla N.R., Sharma V. Random and Gene Specific Amplification as Tool for Phylogenetic Assessment: A Study of HIV Subjects From North India. *International Journal of Science and Technology*. 2012, 1: 384-390.
- Sharma A.K., Sharma V.N., Kalla N.R., Singh A. Correlation of Liver Function Tests with HIV/AIDS in North Indian Population. *International Journal of Science and Advanced Technology*. 2012, 2: 38-44.
- Sharma A.K., Singh A., Garg N., Sirohi S., Sharma V. Intellectual Property Rights: Perspectives in HIV/AIDS Management. Presented in workshop on "*Intellectual Property Rights and Management for Engineering & Technology disciplines*" at NCCE, Israna (March 30-31, 2012).
- Sirohi S., Srivastava V., Garg N., Sharma A.K., Sirohi S.P.S. A review on Polyhydroxyalkanoates: Production and degradation in waste environment through nanobiotechnology. Presented in "*National Conference on Nanosciences and Nanobiotechnology: Present and future Prospectives"* at MIET, Meerut (November 26, 2011).
- Garg N., Sirohi S., Singh A., Sharma A.K., Sirohi S.P.S., Gaurav S.S. Intellectual Property Rights in Nanobiotechnology. Presented in "*National Conference on Nanosciences and Nanobiotechnology: Present and future Prospectives"* at MIET, Meerut (November 26, 2011).
- Sharma A.K., Sharma V.N. HIV/AIDS - related opportunistic infections among people in North India. Presented in "*National Conference on Emerging Trends in Life Sciences Research"* (ETLSR-2009) at BITS, Pilani (March 6-7, 2009).

TRAINING/ WORKSHOP/ CONFERENCES ATTENDED (Last 5 Years)

- AICTE sponsored Two weeks SDP on "**New Frontiers in Molecular Biology"** in December, 2011, at *Meerut Institute of Engineering and Technology (MIET),* Meerut.
- National Conference and Workshop on **"Nanosciences and Nanobiotechnology: Present and future Prospectives"** in November, 2011 at *Meerut Institute of Engineering and Technology (MIET),* Meerut.
- Seminar on **"Intellectual Property and Innovation Management in Knowledge Era"** by National Research Development Corporation (NRDC) in September, 2011, at *Meerut Institute of Engineering and Technology (MIET),* Meerut.
- AICTE sponsored Two weeks SDP on "**Nanotechnology"** in June, 2011, at *Vivekanandha College of Engineering for Women*, Tiruchengode.
- Advanced workshop on **"Innovation in Teaching Methodology"** by **Wipro Mission 10X** in May, 2010, at *Bharat Institute of Technology (BIT),* Meerut.
- Five days workshop on **"Innovation in Teaching Methodology" including "High Impact Teaching Skills"** by **Wipro Mission 10X** and **Dale Carnegie Training** in November, 2009, at *Meerut Institute of Engineering and Technology (MIET),* Meerut.
- ICMR sponsored CME on **"Hospital Associated Infections"** in October, 2009, at *SMC, Meerut*.
- ICMR sponsored Training Course on **"Application of Research Techniques in Reproductive Biomedicine"** in May, 2009, at *Meerut Institute of Engineering and Technology (MIET),* Meerut.
- **"National Conference on Emerging Trends in Life Sciences Research"** in March, 2009, at *Birla Institute of Technology and Science (BITS),* Pilani.
- One week programme on "**Biosciences & Bionanotechnology"** in February, 2009 at *National Institute of Technical Teacher's Training and Research (NITTTR)*, Chandigarh.
- **"National Seminar on Apoptosis-Recent Developments"** in February, 2007, at *Meerut Institute of Engineering and Technology (MIET),* Meerut.

PROFESSIONAL MEMBERSHIPS

- Indian Society for Technical Education, New Delhi, India.
- Association of Biotechnology and Pharmacy, Guntur (A.P.), India.
- Indian Council for Cultural Relations, New Delhi, India.

Place : New-Delhi
Date : 12-12-2012 **(Ajay Kumar Sharma)**