DYSLIPIDEMIA IN TREATMENT NAÏVE HIV

Dissertation submitted for

DOCTOR OF MEDICINE

Branch I – GENERAL MEDICINE

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CHENNAI, TAMILNADU.

CERTIFICATE

This is to certify that the dissertation entitled **"DYSLIPIDEMIA IN TREATMENT NAÏVE HIV"** is the bonafide work of **Dr.SIVAKUMAR V** in partial fulfillment of the university regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for **M.D General Medicine Branch I** examination to be held in **April 2015**.

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DECLARATION

I, **Dr.SIVAKUMAR V**, solemnly declare that, this dissertation "**DYSLIPIDEMIA IN TREATMENT NAÏVE HIV**" is a bonafide record of work done by me at the Department of General Medicine, Government Rajaji Hospital, Madurai, under the guidance of **Dr.C.DHARMARAJ M.D**., **DCH**. Professor, Department of General Medicine, Madurai Medical College, Madurai.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the award of Degree of Doctor of Medicine (M.D.), General Medicine Branch-I, examination to be held in April 2015.

Place: Madurai

Date: **Dr.SIVAKUMAR V**

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DYSLIPIDEMIA IN TREATMENT NAÏVE HIV

INTRODUCTION

ABSTRACT

The natural history of human immunodeficiency virus (HIV) infection changes with the use of highly active antiretroviral therapy (HAART) through reduction in risks of death associated with the condition and improvement of the quality of life of people living with the infection. Abnormalities of lipid metabolism are common in HIV infected patients and tend to be accentuated in those receiving highly active antiretroviral therapy (HAART). The study was conducted to describe the pattern of lipid profile among treatment naïve-HIV positive patients.

Aims and objectives:

To study the derangement of lipid profile in treatment naïve HIV

Methods:

Data were collected from 100 normotensive, non-diabetic and non-obese treatment and 100 age and sex matched healthy controls. The study was carried out at ART center in Govt.Rajaji Hospital, Madurai from December 2013 to July 2014.

Results:

The study observed a significant increased level of triglycerides and low density lipoprotein cholesterol and a significant decreased level of total cholesterol and high density lipoprotein cholesterol.

Conclusion:

HIV-I replication alone without any influence of human genetic factors and antiviral drugs induces changes in serum lipid profile which could be used to determine HIV-infected persons with high risk of Myocardial infarction before enrollment for HAART.

INTRODUCTION

The natural history of human immunodeficiency virus (HIV) infection changes with the use of highly active antiretroviral therapy (HAART) through reduction in risks of death associated with the condition and improvement of the quality of life of people living with the infection. Abnormalities of lipid metabolism are common in HIV infected patients and tend to be accentuated in those receiving highly active antiretroviral therapy (HAART). The study was conducted to describe the pattern of lipid profile among treatment naïve-HIV positive patients.

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REVIEW OF LITERATURE

AIDS has its root in Africa, because some Simian immunodeficiency viruses are closely associated with HIV-1 & HIV-2, a relative counterpart of virus in an African Monkey Sooty-mangabey. So, HIV-2's relation to the Sooty-Mangabey is the reliable evidence for animal to man transfers of HIV.

It's very difficult to exactly pin down the likely source of HIV-1. The closest Simian virus to HIV-1 discovered till date has been found in some Chimpanzees. Even though, it is very difficult to prove that HIV has its origin from Primates, it has been known to infect humans.

In humans, an adult male, who lived in the Democratic Republic of Congo, was the very first evidence for HIV infection.

In 1959, researchers have gained success in separating the virus from a Plasma sample, which was taken from the man. They strongly believed that the origin of this strain may be from 1940s or 1950s and it should have spread among humans, a decade or much earlier.

In 1981, cases of very rare opportunistic infections, Pneumocystis causing pneumonia and an uncommon skin tumor of endothelial cell origin Kaposi Sarcoma were reported in New York and California in epidemic proportions among previously healthy young homosexual and bisexual men, who were not

known to be predisposed previously to these diseases. With the rapidly increasing report of such cases, it was soon recognized that other life threatening infections & neoplastic diseases were also observed and found to be attributed to an unexplained defect in cell mediated immunity common to each of these patients.

In early 1982, the group of disease entities was named the Acquired Immuno Deficiency Syndrome.

In June 1982, a group of cases among gay men in Southern California has suggested that sexually transmitted infections agent may be the etiological agent and initially, the syndrome was named "GRID" (gay related immune deficiency).

At the same time, the disease was reported among hemopiliacs & in female sexual contacts of infected men suggesting transmission through sexual route and blood also.

By August 1982, the gay related immune deficiency was emerged to new CDC – coined term AIDS (Acquired Immuno Deficiency Syndrome) and the CDC has revised this term to add various syndromes acknowledged as manifestations of advanced HIV disease in September 1982.

In May 1983, Luc montagmier and his colleagues (Paster institute - Paris) isolated a retrovirus from AIDS patient presented with generalised

lymphadenopathy and named it as Lymphadenopathy Associated Virus (LAV) which was similar but different from HTLV-1 & HTLV-2.

In May 1984, Robert C Gallo and colleagues (National Institute of Health - Bethesdo) isolated a retrovirus from AIDS patient, similar to HTLV and called it HTLV-III

Cloning and molecular characteristics of genetic materials proved that both the viruses were similar and consistent isolation from patients of different origin, with higher degree of tropism of CD4 lymphocyte and isolation of similar Simian virus causing AIDS in Macaques proved that HTLV-III/LAV was the etiological agent of AIDS.

Screening tests for HIV in blood donors in industrialized countries was started in 1984.

In 1986, centers for disease control provided working definition of AIDS and HTLV-III/LAV were renamed as HIV in Viral Taxonomy by International committee.

Antiretroviral therapy:

In the history of medicine, the advancement of antiretroviral therapy had been one of the most impressive progression.

The years between 1987-1990 brought in trust and some earliest advancements using monotherapy but when the results of the study had arrived, not only patients but also researchers had immersed in to a depression which endured for many years. In 1985, Zidovudine was tested among humans and in March 1987, it was introduced as a treatment with great expectations. Initially it didn't appear to be most effective.

Then the preliminary results of the European – Australian DELTA study and the American ACTG 175 study gained attention. It became apparent that combination therapy with two nucleoside analogs was more effective than single agent therapy. Indeed the differences made on the clinical endpoints (AIDS, death) were highly significant. Both studies showed that it was potentially great important to immediately start treatment with two nucleoside analogs as opposed to using the drugs sequentially.

Epidemiology:

The prevalence of HIV/AIDS varies to a great extent from region to region, from nation to nation and from continent to continent. The joint UN program on HIV/AIDS provides considerably the best and broad overview. The yearly AIDS epidemic update of UNAIDS describes the recent advancements in the global

HIV/AIDS epidemic with maps and regional sum-ups. It inquires recent trends in the epidemic's evolution and renders the latest estimates of the epidemic's scope.

Global Trends:

From the beginning of the epidemic, about 75 million people have been infected with HIV virus and over 36 million people died of HIV whereas in 2012 alone, about 1.6 million people across the world have died due to AIDS related disorders. At the end of 2012, 35.3 million (32.2 – 38.8 million) people were living with HIV globally. About 0.8% of adults aged between 15-49 years are living with HIV worldwide, even if the burden of the epidemic pursues to vary substantially between nations and regions. The most severely affected region with nearly 1 in every 20 people living with HIV is Sub – Saharan Africa, which accounts for 71% among people living with HIV worldwide.

In low and middle income countries, about 9.7 million people infected by HIV had access to antiretroviral therapy in 2012, which indicates about 61% of people were qualified for medication under 2010 WHO recommendation and around 34% of people qualified under 2013 WHO guidelines.

India:

By 2012, the estimated number of HIV infected people was 0.3% of total population aged between 15-49 years with HIV related deaths of 11 per 10,00,00

population. ART coverage among people with HIV infection eligible for ART is 44-58%.

Districts, which has HIV prevalence more than 3% in Antenatal clinic attendees were Prakasam, Mahbubnagar, Nizamabad and West Godavari in Andhra Pradesh, Hassan and Belgaum in Karnataka, Chandrapur and Sanglii in Maharashtra, Wehrul in Manipur, Ganjam in Orissa, Tuensang in Nagaland, Ganganagar in Rajasthan, Salem and Namakkal in Tamil Nadu. Districts which have HIV prevalence more than 15% in sexually transmitted disease clinic attendees were Khammam, Prakasam, Krishna, Vishakapatnam, Hyderabad, Warangal and Chitoor in Andhra Pradesh, Sangli, Mumbai and Nagpur in Maharashtra, Bellary in Karnataka, Tirunelveli and Madurai in Tamil Nadu, Ahmedabad in Gujarat.

Virology:

HIV

Taxonomy – Human Immunodeficiency Virus

Family – Retrovirida

Subfamily – Lentivirinae

Genus – Lentivirus

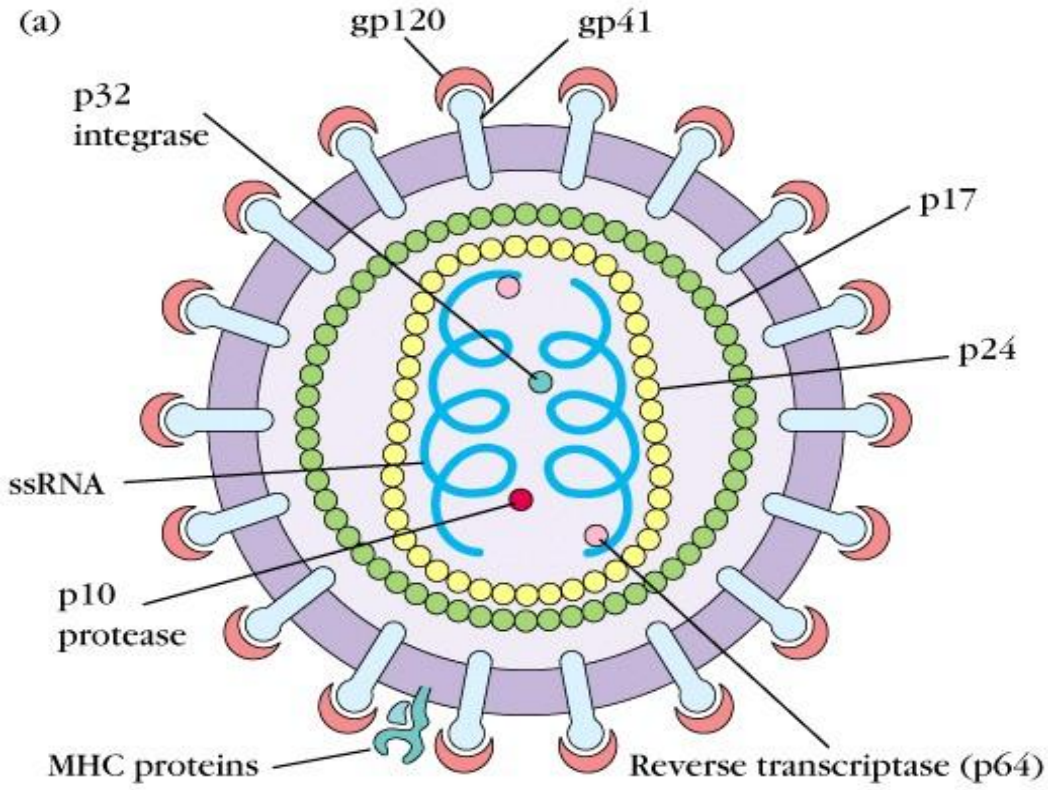

Fig.1: Structure of HIV

Subtype C of M group of HIV-I is the most common form prevalent worldwide and in India. Subtypes of HIV seen in India are given below:

- HIV – I virus 98%
- HIV – II virus <2%
- Subtype of HIV –I virus
  - 95% Class C
  - 3% Class A
  - Rest Class B

Morphology:

HIV, like other retroviruses has an icosahedral structure with multiple external spikes. The virus has a bilipid layer of envelope within which host proteins like major histocompatability complex antigens are incorporated and spikes are formed by glycoprotein 120(gp-120) which is linked non-covalently to transmembrane glycoprotein 41 (gp-41). Inside the envelope is a cone shaped capsid made of p24 viral protein, which contains 2 single stranded RNA.

HIV Gene	Gene product	Function
gag	p17	Matrix protein
	p24	Capsid protein
	p7	Nucleocapsid promotes RNA dimerization and encapsidation
	p6	Not known, role in budding of virus
env	gp160	Precursor of gp120 and gp41
	gp120	Surface envelope glycoprotein for binding to CD4 molecule
	gp41	Transmembrane glycoprotein results in membrane fusion of virus envelope with CD4 lymphocyte
pol	Protease	Cleaves gag and pol gene products
	Reverse Transcriptase	Catalyze reverse transcription of HIV RNA to double stranded DNA

HIV Gene	Gene product	Function
	Integrase	Integrates viral DNA in to host cell chromosome
Tat	Tat	Enhances transcription of viral RNA
Rev	Rev	Cytoplasmic transport of unspliced RNA
Vpu	Vpu	Promotes selective degradation of CD4 also facilitates virion release from host cell
Vif	Vif	Stabilizes the virion upon entry in to host cell
Vpr	Vpr	Induces arrest of host cell in G2 phase
Nef	Nef	Down regulates CD4 and major histocompatibility complex expression

Table 1: HIV Gene, Proteins and Their Function.

Transmission:

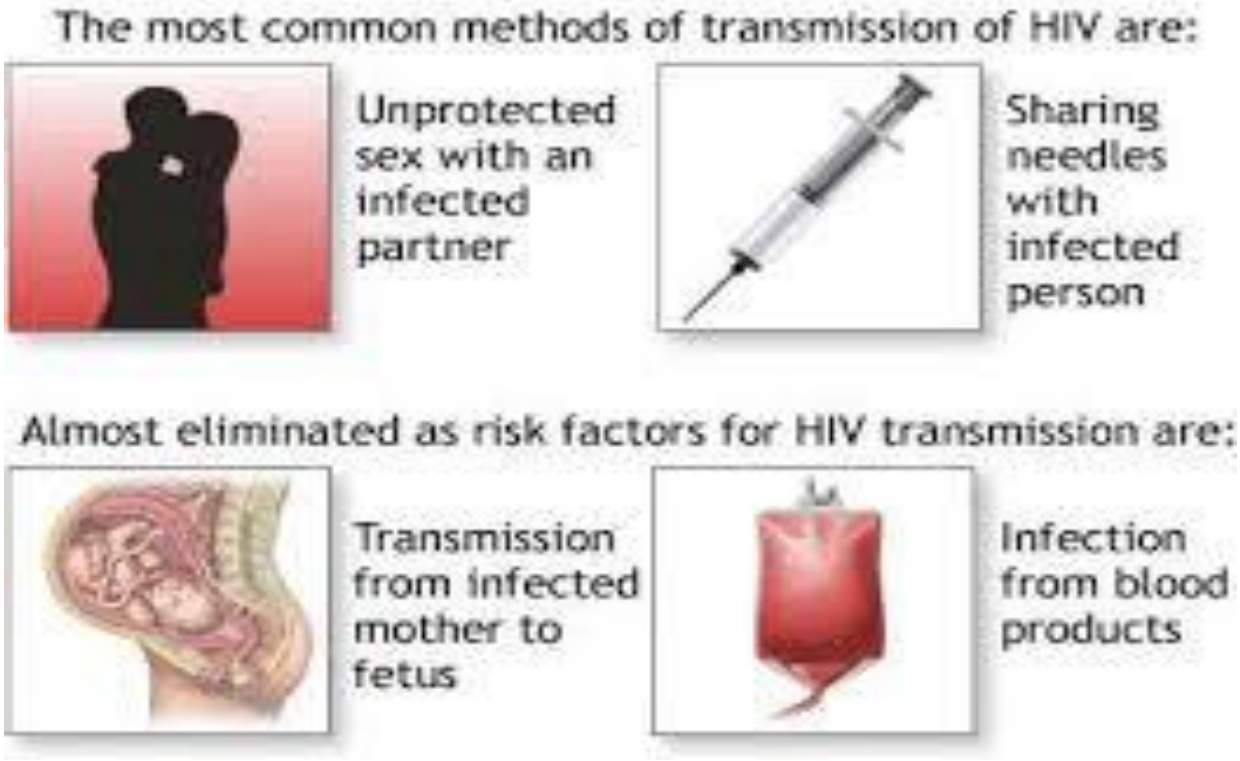

Fig. 2: Various Modes of HIV Transmission

Sexual Transmission:

The global prevalence of AIDS epidemic is mainly due to the sexual transmission of HIV-I and the degree by which it can be reduced determines the future of AIDS expansion globally. Among heterosexuals, sexual transmission is the most predominant way of spread of HIV. HIV is sexually transmitted by penile vaginal intercourse and penile anal intercourse and sometimes through fellution. Vaginal intercourse could transmit the disease to either male or female but the risk

is more to female partner. Meta-analysis from various studies regarding HIV transmission found that use of barrier methods like condom has an efficiency of 69% and use of Zidovudine resulted in reduced amount of retrovirus in semen.

IV drug use related HIV infection:

HIV transmission in injection drug abusers happens mostly through contamination of injection paraphernalia by HIV infected blood, and this is re-used by an uninfected drug users but this mode of transmission is greatly reduced after the introduction of disposable syringes. However sharing the same injection is a usual practice with injection drug abusers all over the world. Sharing of syringes, needles, and some other injection equipment is the foremost risk factor.

Vertical transmission:

HIV infections in children are mainly due to Perinatal transmission from infected mother.

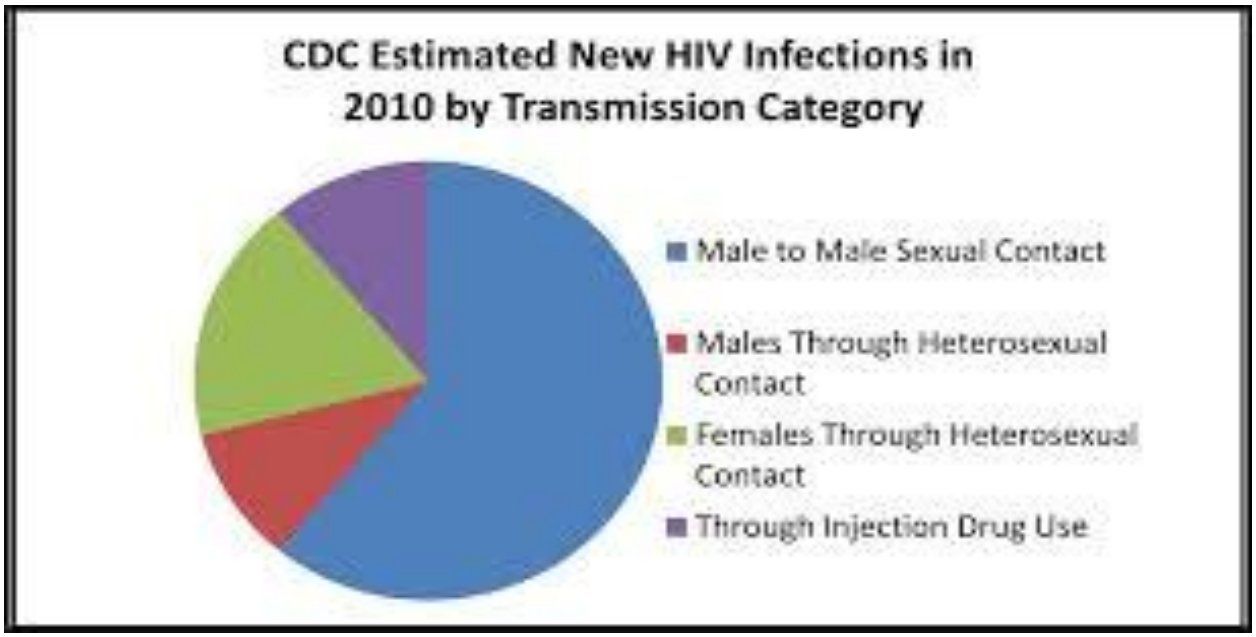

Fig. 3: HIV Infections

Transmission by other routes:

Blood products derived from an infected person and processed in to a blood component transmit HIV. i.e., fresh frozen plasma, whole blood packed red cells, platelets and cryoprecipitate.

HIV disease: Pathogenesis:

Chronicity is the characteristic of HIV infection and has several targets including CD4+Tcells dendritic cells and macrophages.

HIV mostly enters the host through the genital mucosa. The viral envelope protein, binds to the CD4 molecule. Interstitial dendritic cells found in

cervicovaginal epithelium as well as adenoidal and tonsillar tissue, the usual first target cells in infections acquired through genito-oral sex.

Viral entry in to these cells is facilitated by different co-receptors. The virus enters macrophage, when gp-1 interacts with the chemokine receptor CCR5 as well as CD4. Macrophage tropic viruses are designated as R5 in comparison to T cell tropic viruses, which are called X4, based upon the CXCR4 receptor on these cells. Patients are relatively resistant to R5 infection if they are homozygous for a deletion in CCR5.

HIV infected cells fuse with CD4 + T cells, leading to spread of the virus. HIV is detectable in regional lymph nodes within two days of mucosal exposure and within 5 days in plasma.

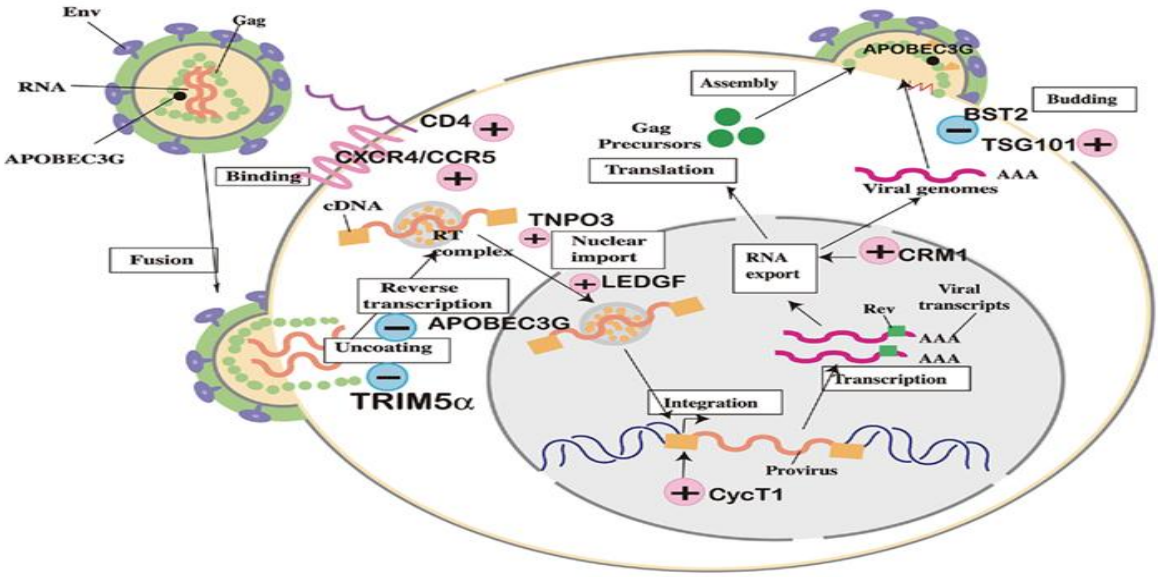

Fig. 4: Replication of HIV

Once virus enters the blood, there is widespread dissemination to organs such as spleen, lymph node as well as brain. Viremia occurs between 5 to 30 days after experimental intra-vaginal HIV exposure.

This initial viremia disseminates the virus to other lymphoid organs. This is followed by persistant active viral replication with progressive CD4 + T cell depletion latent stage. Hyperplasia of germinal centers with copious amount of virion trapped in the follicular dendritic cells in lymph nodes. These trapped virions serve as a constant source of infection to CD4 + T lymphocytes in the parafollicular area. These trapped virions provide a constant source of cellular activation, which results in secretion of IL-1ß, TNF-α, IL-6, which up regulate viral replication and expression in infected cells. The architecture of lymph node, the thymus and other lymphoid organs is lost as the disease progress.

During initial periods of HIV infection, patients have more susceptible CD4 + T cells and no HIV–specific immune response. Viral infection is therefore rapid. Plasma HIV RNA levels reach more than 10^7 copies/ml. p24 antigen levels exceed 100 pg/ml.

While HIV specific immunity is evolving, primarily due to the emergence of virus specific CD8+ cytotoxic T lymphocytes, plasma RNA levels fall precipitously by 2 to 3 logs and symptoms of acute retroviral syndrome resolve.

Plasma HIV RNA levels will stabilize at a particular set-point within six months of infection in the absence of antiretroviral therapy.

A few HIV – positive individuals have normal CD4 counts and low or undetectable plasma viraemia even in the absence of appropriate therapy are classified as long-term non-progressors. A new term for such individuals is "Controller" implying their ability to control viral replication without antiretroviral therapy.

Chronicity of Infection:

HIV has an extraordinary ability to mutate especially v_3 region of gp-120, hence escapes from immune defense mechanisms like neutralizing antibodies. Cytolytic CD8 + T lymphocytes need CD4 + lymphocytes help in induction and maintenance of Cytolytic CD8 + T cell responses. With depletion of CD4 + T cells, the functions of CD8 + T cells are reduced. Due to overwhelming exposure of viral antigen, CD8 + T lymphocytes gradually reduce in number. A large pool of latently infected cells is present which are quiescent and activation may express the HIV virus.

Role of Chronic Cellular activation:

Persistent infection causes hypergammaglobulinemia due to hyperactivation of B-lymphocytes. Activation of CD4 + T lymphocytes, monocytes and CD8 + T

lymphocytes results in increase in proinflammatory cytokines. Proinflammatory cytokines increase the expression of HIV in infected cells.

Role of Cytokines in HIV Pathogenesis:

Proinflammatory cytokines like tumor necrosis factor α (TNF - α) , Interleukin - 1ß, Interleukin 6(IL-6) are the most potent inducers of HIV expression. (NF-KB) the transcriptional activators of HIV expression are activated by TNF α. Interferon α and ß suppress HIV replication. Interleukin 10 inhibits multiplication of HIV in acutely infected monocytes by blocking secretion of TNF α and IL-6.

Chemokines – RANTES, macrophage inflammatory protein (MIP) - 1α and MIP -1ß inhibit infection by and spread of macrophage tropic/R5 HIV strains, which block the CCR5 co-receptor. They are secreted by natural killer cells. Stromal cell derived factor (SDF)-1 inhibits infection by and spread of T cell tropic/X4 HIV strains which block CXCR4 co-receptor on the target cells.

In HIV infection, Type I T-Helper response that upregulates cellular immunity is reduced IL-2 and IL-12 stimulate lytic activity and proliferation of Cytotoxic T lymphocytes and natural killer cells.

HIV – infected individuals have loss of IL-2 receptors and reduced ability to produce IL-12 and Interleukin 2.

Dysfunction and Depletion of CD4 + T lymphocytes:

The hallmark of HIV disease is dysfunction of CD4 + T cells, both qualitative and quantitative defects are seen. Some early abnormalities noted are loss of response to remote recall antigens like influenza, tetanus toxoid etc. This is followed by loss of proliferative response of T cells to alloantigen and later to mitogenic stimulus.

Mechanism of CD4 + T cell depletion & Dysfunction:

A) Direct Mechanisms:

Single cell killing due to direct infection with HIV is less likely to be a major contributor for immune dysfunction at least in early HIV infection. The Proportion of HIV infected CD4 + T cells in peripheral blood ranges from 1 to 10,000 in early infection to 1 in 100 cells in advanced stages.

Accumulation of Unintegrated Viral DNA

High intracellular levels of Viral DNA, interferes with signal transduction

Viral budding results in loss of plasma membrane integrity.

HIV infected cells may be killed by virus specific immune responses.

B) Indirect Mechanisms:

Syncytium formation results from fusion of cell membranes of infected and uninfected CD4 + T cells have been observed in vitro.

Molecular mimicry between Class II MHC and gp-120 results in auto antibodies to self MHC determinants and elimination of these cells.

Elimination of uninfected CD4 + T cells coated with HIV gp-120 by HIV specific immune response called “Innocent bystander killing”.

Apoptosis:

Gp-120 and CD4 interaction causes a state of altered cell activation wherein a second activation signal like antigen binding of T cell receptor triggers further apoptosis. Viral Tat protein also up regulates Fas ligand (CD95) which results in apoptotic cell death.

Infection of thymocytes and CD34 + bone marrow progenitor cells leads to depletion of T cell precursors. Thymic microenvironment I disrupted.

Abnormalities of other cells of immune system:

CD8 + T lymphocytes:

Initially there will be a robust cytotoxic response but this wanes gradually in later stage. These cells lose functional capability of cytolytic activity and develop abnormal phenotype in advanced HIV disease. CD4 + T helper lymphocytes are necessary for inducing and maintaining cytotoxic lymphocyte response.

B lymphocytes:

Polyclonal B cell activation by HIV or its products like gp-41 results in hypergammaglobulinemia. B cells are also functionally defective and respond poorly to immunization. These defects make the person more susceptible to certain bacterial infections.

Monocytes/Macrophages:

Though HIV can replicate in monocyte lineage cells, it has little cytopathic effect on them. Defects in antigen presentation and decreased secretion of cytokines are functional deficits seen in Macrophages/Monocytes.

Natural killer cells:

They are normal in function and number and serve as important source of HIV inhibitory chemokines.

CLINICAL CATEGORIES OF HIV INFECTION

CLINICAL EVENT	CLINICAL DIAGNOSIS	DEFINITIVE DIAGNOSIS
CLINICAL STAGE 1		
Asymptomatic	No HIV-related symptoms reported and no signs on examination	Not applicable
Persistent generalized lymphadenopathy	Painless enlarged lymph nodes >1cm in two or more non-contiguous sites (excluding inguinal) in the absence of known cause and persisting for three months or more	Histology
CLINICAL STAGE 2		
Moderate unexplained weight loss (<10% of body weight)	Reported unexplained involuntary weight loss in pregnancy failure to gain weight	Documented weight loss <10% of body weight
Recurrent upper respiratory tract infections (current event plus one or more in last six-month period)	Symptom complex, such as unilateral face pain with nasal discharge (sinusitis), painful inflamed eardrum (otitis media) or tonsillopharyngitis without features of viral infection (such as coryza or cough)	Laboratory studies where available, such as culture of suitable body fluid

Herpes zoster	Painful vesicular rash in dermatomal distribution of a nerve supply, does not cross the midline	Clinical diagnosis
Angular cheilitis	Splits or cracks at the angle of the mouth not due to iron or vitamin deficiency, usually respond to antifungal treatment	Clinical diagnosis
Recurrent oral ulcerations (two or more episodes in last six months)	Aphthous ulceration, typically painful with a halo of inflammation and a yellow-grey pseudomembrane	Clinical diagnosis
Papular pruritic eruption	Papular pruritic lesions, often with marked post-inflammatory pigmentation	Clinical diagnosis
Seborrhoeic dermatitis	Itchy scaly skin condition, particularly affecting hairy areas (scalp, axillae, upper trunk and groin)	Clinical diagnosis
Fungal nail infections	Paronychia (painful red and swollen nail bed) or onycholysis (separation of the nail from the nail bed) of the fingernails (white discoloration – especially involving proximal part of nail plate – with thickening and separation of the nail from the nail bed)	Fungal culture of the nail or nail plate material

CLINICAL STAGE 3		
Unexplained severe weight loss (more than 10% of body weight)	Reported unexplained involuntary weight loss (>10% of body weight) and visible thinning of face, waist and extremities with obvious wasting or body mass index <18.5 kg/m^2; in pregnancy, the weight loss may be masked	Documented loss or more than 10% of body weight
Unexplained chronic diarrhea for longer than one month	Chronic diarrhea (loose or watery stools three or more times daily) reported for longer than one month	Three or more stools observed and documented as unformed, and two or more stool tests reveal no pathogens
Unexplained persistent fever (intermittent or constant and lasting for longer than one month)	Fever or night sweats for more than one month, either intermittent or constant with reported lack of response to antibiotics or antimalarial agents, without other obvious foci of disease reported or found on examination; malaria must be excluded in malarious areas	Documented fever $>37.5^0C$ with negative blood culture, negative Ziehl-Nielsen stain, negative malaria slide, normal or unchanged chest X-ray and no other obvious focus of infection
Oral candidiasis	Persistent or recurring creamy white curd-like plaques that can be scraped off (pseudomembranous) or red patches on tongue,	Clinical diagnosis

	palate or lining of mouth, usually painful or tender (erythematous form)	
Oral hairy leukoplakia	Fine white small linear or corrugated lesions on lateral borders of the tongue that do not scrape off	Clinical diagnosis
Pulmonary tuberculosis (current)	Chronic symptoms:(lasting more than 2-3 weeks) cough, haemoptysis, shortness of breath, chest pain, weight loss, fever, night sweats, and no clinical evidence of extrapulmonary disease Discrete peripheral lymph node M.tuberclosis infection (especially cervical) is considered a less severe form of extrapulmonary tuberclosis	One or more sputum smear positive for acid-fast bacilli and/or radiographic abnormalities consistent with active tuberculosis and/or culture positive for Mycobacterium
Severe bacterial infection (such as pneumonia, meningitis, empyema, pyomyositis, bone or joint infection, bacteraemia and severe pelvic inflammatory disease)	Fever accompanied by specific symptoms or signs that localize infection and response to appropriate antibiotic	Isolation of bacteria from appropriate clinical specimens
Acute necrotizing ulcerative gingivitis or necrotizing	Severe pain, ulcerated gingival papillae, loosening of teeth,	Clinical diagnosis

ulcerative periodontitis	spontaneous bleedin, bad odour and rapid loss of bone and/or soft tissue	
Unexplained anaemia(<8/dl), neutropenia(<0.5x10^9 per liter) or chronic(more than one month) thrombocytopenia(<50x10^9per liter)	Not presumptive clinical diagnosis	Diagnosed on lab testing and not explained by other non HIV conditions; not responding to standard therapy with hematinics, antimalarial agents or anthelmintic agents as outlined in relevant national treatment guidelines, WHO integrated management of childhood illness guidelines or other relevant guidelines
CLINICAL STAGE 4		
HIV wasting syndrome	Unexplained involuntary weight loss (>10% baseline body weight), with obvious wasting or body mass index< 18.5 PLUS Unexplained chronic diarrhea(loose or watery stools three or more times daily) reported for longer than one month	Documented weight loss >10% of body weight PLUS Two or more unformed stools negative for pathogens OR Documented temperature

	OR Reports of fever or night sweats for more than one month without other cause and lack of response to antibiotics or antimalarial agents; malaria should be excluded in endemic areas	of >37.5^{o} C with no other cause o disease, negative malaria slide and normal or unchanged chest X ray
Pneumocystis pneumonia	Dyspnoea on exertion or nonproductive cough o recent onset (within the past three months), tachypnoea and fever AND Chest X-ray evidence of diffuse bilateral interstitial infiltrates AND No evidence of bacterial pneumonia; bilateral crepitations on auscultation with or without reduced air entry	Cytology or immunofluorescent microscopy of induced sputum or bronchoalveolar lavage or histology of lung tissue.
Recurrent severe bacterial pneumonia	Current episode plus one or more previous episodes in the past six months; acute onset (<2	Positive culture or antigen test of a compatible organism

	weeks) of severe symptoms (such as fever, cough, dyspnoea and chest pain) PLUS new consolidation on clinical examination or chest X-ray; response to antibiotics	
Chronic herpes simplex virus infection (orolabial, genital or anorectal) of more than one month or visceral infection of any duration	Painful, progressive anogenital or orolabial ulceration; lesions caused by recurrence of herpes simplex virus infection and reported for more than one month. History of previous episodes. Visceral herpes simplex virus requires definitive diagnosis	Positive culture or DNA(by polymerase chain reaction) of herpes simplex virus or compatible cytology or histology
Oesophageal candidiasis	Recent onset of retrosternal pain or difficulty on swallowing(foods and fluids) together with oral candida	Macroscopic appearance at endoscopy or bronchoscopy, or by microscopy or histology
Extrapulmonary tuberculosis	Systemic illness (such as fever, night sweats, weakness and weight loss). Other evidence for extrapulmonary or disseminated tuberculosis varies by site, such as pleura, pericardia, meninges, mediastinum or abdominal Discrete peripheral lymph	M. tuberculosis isolation or compatible histology from appropriate or radiological evidence of miliary TB(diffuse uniformly distributed small military shadows or micronodules on CXR)

	node mycobacterium tuberculosis infection (especially cervical) is considered a less severe form of extrapulmonary tuberculosis	
Kaposi sarcoma	Typical gross appearance in skin or oropharynx of persistent, initially flat, patches with a pink or violaceous colour, skin lesions that usually develop into plaques or nodules	Macroscopic appearance at endoscopy or bronchoscopy or by histology.
Cytomegalovirus disease(other than liver, spleen or lymph node)	Retinitis only; may be diagnosed by experienced clinicians. Typical eye lesions on funduscopic examination; discrete patches of retinal whitening with distinct borders, spreading centrifugally, often following blood vessels, associated with retinal vaculitis, hemorrhage and necrosis	Compatible histology or cytomegalovirus demonstrated in CSF by culture or DNA by PCR
CNS toxoplasmosis	Recent onset of a focal nervous system abnormality consistent with intracranial disease or reduced level of consciousness AND response within 10 days to specific therapy	Positive serum toxoplasma antibody AND (if available) single or multiple intracranial mass lesion on neuroimaging (computed tomography or magnetic resonance imaging)
HIV encephalopathy	Disabling cognitive and/or motor dysfunction	Diagnosis of exclusion: and (if available)

	interfering with activities of daily living, progressing over weeks or months in the absence of a concurrent illness or condition other than HIV infection that might explain the findings	neuroimaging (computed tomography or magnetic resonance imaging)
Extrapulmonary cryptococcosis (including meningitis)	Meningitis: usually subacute, fever with increasing severe headache, meningism, confusion, behavioural changes that respond to cryptococcal therapy	Isolation of Cryptococcus neoformans from extrapulmonary site or positive cryptococcal antigen test on cerebrospinal fluid or blood
Disseminated non-tuberculous myobacterial infection	No presumptive clinical diagnosis	Diagnosed by finding atypical myobacterial species from stool, blood, body fluid or other body tissue, excluding the lungs
Progressive multifocal leukoencephalopathy	No presumptive clinical diagnosis	Progressive nervous system disorder (cognitive dysfunction, gait/speech disorder, visual loss, limb weakness and cranial nerve palsies) together with hypodense white matter lesions on neuro-imaging or positive polyomavirus JC positive polymerase chain reaction on cerebrospinal fluid
Chronic cryptosporidiosis (with diarrhea lasting	No presumptive clinical	Cysts identified on modified Ziehl-Nielsen

more than one month)	diagnosis	stain microscopic examination of unformed stool
Chronic isosporiasis	No presumptive clinical diagnosis	Identification of Isospora
Disseminated mycosis (such as coccidiomycosis, histoplasmosis or penicilliosis)	No presumptive clinical diagnosis	Histology, antigen detection or culture from clinical specimen or blood culture
Recurrent non-typhoid Salmonella bacteraemia	No presumptive clinical diagnosis	Blood culture
Lymphoma (cerebral or B-cell non-Hodgkin)	No presumptive clinical diagnosis	Histology of relevant specimen or, for central nervous system tumours, neuroimaging techniques
Invasive cervical carcinoma	No presumptive clinical diagnosis	Histology or cytology
Visceral leishmaniasis	No presumptive clinical diagnosis	Diagnosed by histology (amastigotes visualized) or culture from any appropriate clinical specimen
HIV-associated nephropathy	No presumptive clinical diagnosis	Renal biopsy
HIV-associated cardiomyopathy	No presumptive clinical diagnosis	Cardiomegaly and evidence of poor left ventricular function confirmed by echocardiography

Table 2: Clinical Categories of HIV Infection

Diagnosis of HIV Infection:

The diagnosis of HIV infection depends as the direct detection/demonstration of antibodies to HIV or one of its components. Generally the antibodies to HIV appear in the circulation 3-12 weeks following infection.

ELISA:

Also referred as Enzyme immuno assay is the standard blood screening test for HIV infection with a sensitivity of >99.5%

Commercial EIA kit that most diagnostic laboratories contains antigens from both HIV-I and HIV-II and thus able to detect either. The fourth generation EIA tests combine detection of antibodies to HIV with detection of p24 antigen to HIV. While EIA is an extremely sensitive test, it is not optimal with specificity especially in studies of low-risk individuals such as volunteer blood donors. Factors that all associated with false positive EIA tests are antibodies to class II antigens such as may be seen following pregnancy, blood transfusion or transplantation, auto antibodies, hepatic disease, recent influenza vaccination and acute viral infections. For these reasons, anyone suspected of having HIV infection based on a inconclusive EIA or positive EIA result must have the result confirmed with a more specific assay such as Western blot.

Western blot:

This is the most commonly used confirmatory test. A Western blot demonstrates antibodies to product of all three of the major genes of HIV (Gag, Pol and Env) are confirmative evidence of HIV infection. While the western blot is an excellent confirmatory test for HIV infection in patients with a positive or indeterminate EIA, it is a poor screening test. In 1993, the U.S. FDA established the criteria for a positive western blot state if antibodies to two of these HIV proteins p24, gp41and gp120/160 were present. In addition, the diagnosis of HIV can be confirmed with p24 antigen capture assay or one of the tests for HIV RNA.

p24 antigen capture assay:

This is the simplest of the direct detection tests in an EIA based format. The p24 antigen capture assay has its greatest use as a screening test for HIV infection in patients suspected of having the acute HIV syndrome as high levels of p24 antigen are seen prior to the development of antibodies during the first few weeks of infection. This test is positive in 50% of patients and detects down to 15pg/ml of p24 protein.

Other Direct Detection tests of HIV:

These tests measure and monitor levels of HIV RNA in the plasma of patients with HIV infection. These assays are predominantly used for this purpose.

HIV RNA by reverse transcriptase PCR:

- PCR amplification of CDNA generated from viral RNA(target amplification)
- Can reliably detect 40 copies/ml of HIV RNA.

HIV RNA by bDNA(branched DNA):

- Measures particle associated HIV RNA in a nucleic acid capture assay employin signal amplification.
- Can reliably detect 50 copies/ml of HIV RNA.

HIV RNA by NASBA (Nucleic acid sequence based amplification):

- Uses the technique of isothermic nucleic acid amplification with internal controls.
- Can reliably detect 80 copies/ml of HIV RNA.

TREATMENT:

General Principles of Patient Management:

Once a diagnosis of HIV infection was made, detailed clinical examinations and lab studies are done to know the severity of disease and to know the baseline parameter of that particular individual.

Initial Evaluation of the Patient with HIV Infection:

- History and physical examination
- Routine chemistry and hematology
- AST, ALT, direct and indirect bilirubin
- Lipid profile and fasting glucose
- CD4+ T lymphocyte count
- Two plasma HIV RNA levels
- HIV resistance testing
- HLA-B5701 screening
- RPR or VDRL test
- Anti-Toxoplasmosis antibody titer
- PPD skin test
- Mini-Mental Status Examination

- Serology's for hepatitis A, hepatitis B, and hepatitis C
- Immunization with pneumococcal polysaccharide; influenza as indicated
- Immunization with hepatitis A and hepatitis B if seronegative
- Counseling regarding natural history and transmission
- Help contacting others who might be infected

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; VDRL, Venereal Disease Research Laboratory; RPR, rapid plasma reagin; PPD, purified protein derivative;

Antiretroviral therapy:

ART drugs can be divided into four categories

- RTIs(Reverse transcriptase inhibitors) .
  - Nucleoside and nucleotide RTIs.
  - Non nucleoside RTIs.
- PIs(Protease inhibitors)
- Integrase inhibitors

- Viral entry inhibitors
  - CCR5 antagonists
  - Fusion inhibitors

Principles of Therapy of HIV infection:

1. Ongoing HIV replication leads to immune system damage and progression to AIDS.

2. Plasma HIV RNA levels indicate the magnitude of HIV replication and the rate of CD4+ T cell destruction. CD4+ T cell counts indicate the current level of competence of the immune system.

3. Rates of disease progression differ among individuals, and treatment decisions should be individualized based on plasma HIV RNA levels and CD4+ T cell counts.

4. Maximal suppression of viral replication is a goal of therapy; the greater the suppression the less likely the appearance of drug-resistant quasispecies.

5. The most effective therapeutic strategies involve the simultaneous initiation of combinations of effective anti-HIV drugs with which the patient has not been previously treated and that are not cross-resistant with antiretroviral agents that the patient has already received.

6. The antiretroviral drugs used in combination regimens should be used according to optimum schedules and dosages.

7. The number of available drugs is limited. Any decisions on antiretroviral therapy have a long-term impact on future options for the patient.

8. Women should receive optimal antiretroviral therapy regardless of pregnancy status.

9. The same principles apply to children and adults. The treatment of HIV-infected children involves unique pharmacologic, virologic, and immunologic considerations.

10. Compliance is an important part of ensuring maximal effect from a given regimen. The simpler the regimen, the easier it is for the patient to be compliant

Classification of ARV Drugs:

Nucleoside/Nucleotide reverse transcriptase inhibitors:

- Lamivudine (3TC)
- Didanosine (DDI)
- Zidovudine (AZT)
- Emtricitabine(FTC)

- Zalcitabine (DDC)
- Tenofovir
- Stavudine (D4T)
- Abacavir

Non Nucleoside RTIs:

- Nevirapine (NVP)
- Delavirdine (DLV)
- Rilpivirine
- Efavirenz (EFV)
- Etravirine

Protease inhibitors:

- Lopinavir (LPV)
- Indinavir (IDV)
- Amprenavir (APV)
- Fosamprenavir (FPV)
- Ritonavir (RTV)
- Atazanvir (ATV)
- Saquinavir (SQV)

- Darvnavir (DRV)
- Tipranavir (TPV)
- Nelfinavir (NFV)

Entry inhibitors:

- Enfuvirtide (ENF)
- Maraviroc (MVC)

Integrase inhibitors:

- Raltegravir (RAR)
- Elvitegravir

Criteria for Starting ART in HIV patients:

1. Acute HIV syndrome

2. Chronic infections

    A. Symptomatic patients includes HIV-associated nephropathy

    B. Asymptomatic patients

    1. CD4+ T cell count <500/L[a]

    2. Pregnancy

3. Post exposure prophylaxis

[a] - controversial area some start treatment regardless of CD4+ count

TREATMENT IN SPECIFIC SITUATIONS:

HIV AND TUBERCULOIS:

- Start Efavirenz based regimen
- Start ART for all patients irrespective of CD4 count
- Start TB treatment first and when it is tolerated start ART and within first 8 weeks

HIV AND PREGNANCY:

WHO stages I and II: start ART at CD4<350 cells/mm^3

WHO stages III and IV: start ART irrespective of CD4 count

HIV + HBV:

If treatment is not indicated for either infection, monitor patient.

If treatment is indicated for HIV, then start with TDF + 3TC/FTC – based regimen along with Efavirenz.

If treatment is indicated only for HBV, then Peg interferon is recommended.

HCV treatment:

- HCV infection increases the risk of developing hepatic toxicity of antiretroviral treatment.
- First start ART and when CD4 rises to >350 cells/m^3, anti-HIV is considered.
- Treatment of HCV is with Peg-interferon & Ribavirin.
- Avoid AZT or Didanosine as they are contraindicated with Ribavirin.

Indications for Changing HAART regimen in HIV patients

- Following the initiation of therapy drop in plasma HIV RNA levels by less than 1 log in 4 weeks
- Significant increase in plasma HIV RNA levels defined as threefold or greater, that is not attributable to intercurrent infections, testing methods
- Continuously decreasing CD4+ T cell count
- Clinically deteriorating patients
- Side effects
- the change should be starting at least two effective drugs except in drug toxicity where single drug substitution is acceptable

Toxicity of Commonly Used Antiretroviral Drugs:

Zidovudine:

Anemia, granulocytopenia, lipid abnormalities, lipoatrophy, hepatomegaly, steatosis, headache, nausea, nail pigmentation, lactic acidosis, hyperglycemia, myopathy.

Stavudine:

Peripheral neuropathy, ascending neuromuscular weakness, hepatomegaly, lactic acidosis, steatosis, lipodystrophy, lipid abnormalities, pancreatitis, hyperglycemia.

Didanosine:

Lactic acidosis, Pancreatitis, nausea, abnormalities on liver LFT, hepatomegaly with steatosis, optic neuritis, peripheral neuropathy, hyperglycemia

Zalcitabine:

Oral ulcers, pancreatitis, peripheral neuropathy, lactic acidosis, steatosis.

Lamivudine:

Flare of hep B co infection after discontinuing drug

Emtricitabine:

Skin discoloration, Hepatotoxicity

Abacavir:

Hypersensitivity reactions especially in HLA-B5701+ persons (can be fatal); nausea, vomiting, fever, rash, loss of appetite, and malaise or fatigue.

Tenofovir:

Renal osteomalacia, Hep B flare after discontinuation in coinfected persons

Delavirdine:

Skin rash, abnormalities in liver function tests

Nevirapine:

Skin rash, hepatotoxicity

Efavirenz:

Potentially teratogenic, rash, elevated liver function tests, lipid abnormalities, drowsiness, abnormal dreams, dysphoria, depression.

Etravirine:

Rash, nausea, hypersensitivity reactions

Rilpivirine:

Nausea, dizziness, somnolence, vertigo, less CNS toxicity and rash than Efavirenz

Protease Inhibitors:

Abdominal pain, abnormal stools, weakness, headache, hyperglycemia, hypertriglyceridemia, hyperuricemia, lipodystrophy.

Enfuvirtide:

Hypersensitivity reactions, Local injection reactions, increased incidence of bacterial pneumonia.

Maraviroc:

Hepatotoxicity, abdominal pain, fever, , musculoskeletal symptoms, cough, rash, dizziness, nasopharyngitis.

Raltegravir:

Rhabdomyolysis, diarrhea, Nausea, headache, CPK elevation, muscle weakness.

Plasma Lipids and Lipoproteins:

There are lot of studies to prove the association between dyslipidemia and atherosclerosis. Current understanding of physiology and pathology of plasma lipids is based mainly on the concept of plasma lipoproteins and the form in which they circulate in the blood.

Chemistry of Lipids:

The lipids constitute a wide range of substances of biological origin. These are soluble in organic solvents and are poorly soluble in water. Metabolically lipids are more closely interrelated, being transported together in the plasma lipoproteins and sharing certain regulatory mechanisms.

Lipids can be classified as follows:

Total Lipids

Simple Lipids	**Complex Lipids**
1. Fatty acids	1. Triglycerides
2. Sterols: cholesterol	2. Cholesterol esters
Steroid hormones	3. Phospholipids

Vitamin D

3. Terpenes(carotenes, Vit-A,E & K)

4. Prostaglandins

4. Sphingolipids

5. Waxes

Fatty Acids:

They are present as such in minute concentrations in plasma and cells. They are constituents of most lipid classes. They contain a carboxylic acid group and hydrocarbon chains are thus aliphatic monocarboxylic acids. The fatty acids are grouped into saturated and unsaturated fatty acids depending on the absence or presence of a double bond respectively. The main saturated fatty acids are palmitic and stearic acids. Most of the fatty acids are carried mainly by albumin. Essential fatty acids are those which cannot be synthesized in the body. Free fatty acids are immediately available energy sources and provide much of the energy requirements of the body. (Normal values range from 250-400 mg/dI).

Cholesterol:

Cholesterol has a steroid structure i.e., perhydro cyclo pentano phenanthrene ring. Cholesterol has twenty seven carbon atoms. Cholesterol is by far the most abundant sterol in human tissues. The adult human body contains about 150gms. It is the precursor of bile acids, steroid hormones and vitamin D and has an important

structural role mainly in cellular but to a small extent also in intracellular membranes.

The tissue cholesterol is largely in 'free' (non-esterified) form, while in plasma; about 60-70% is present as cholesteryl esters. In man this esterification occurs in plasma, due to the circulating enzyme, lecithin cholesterol acyl transferase (LCAT). The fatty acid is transferred from the 2nd position of lecithin to cholesterol. For routine clinical purposes, the estimation of total cholesterol is adequate. (Normal values range from 150-200 mg/dl).

Triglycerides:

(Neutral fats or fats) are compounds of one molecule of glycerol united by ester bonds to three molecules of fatty acids. If only one or two of the three hydroxyl groups of glycerol is esterified then they are called mono or diglycerides respectively. Triglycerides are an important energy store. Plasma triglycerides are derived from two sources. Exogenous or dietary triglycerides are derived from food and circulate in the plasma in the form of chylomicrons. These are large, low density particles which are formed in intestinal epithelial cells and appear in the plasma soon after a meal. Chylomicrons deliver dietary triglycerides to the adipose and other tissues. In normal individuals, they are entirely cleared from the plasma within 12 hours of fasting.

Endogenous triglycerides are derived primarily from hepatic conversion of carbohydrate and amino acids. Eighty percent of it circulates in plasma in the form of very low density lipoproteins (VLDL), which transport triglycerides to adipose and other tissues. As triglycerides are removed, intermediate density lipoproteins is formed, some of which undergoes catabolism in the liver to low density lipoprotein (LDL) particles. A small proportion (approximately 15 percent) of plasma triglycerides are carried in LDL and a tiny fraction is contained in the high density lipoprotein (HDL) moieties.

The 1984 Consensus Panel divided plasma triglyceride levels into three categories: “normal” was defined as a TG level less than 250 mg/dl and HTG was dichotomized into “borderline hypertriglyceridemia” (250 to 500 mg/dl) and “true hypertriglyceridemia” (>500 mg/dl). Levels of TG greater than 1000 mg/dl are usually due to chylomicronemia and are believed to be able to cause recurrent abdominal pain and pancreatitis.

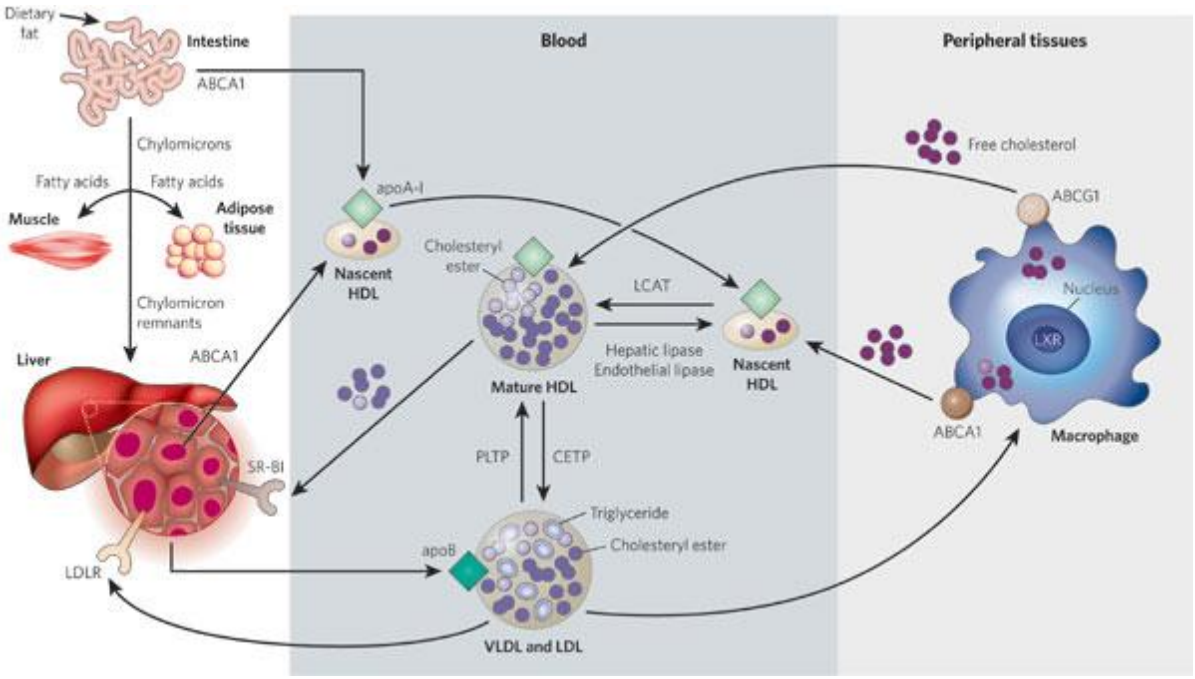

Fig. 5: Exogenous and Endogenous Transport of Lipids

Elevated triglycerides are clinically important owing to their direct relationship to pancreatitis, and their association with glucose intolerance and renal and hepatic disease. Triglyceride measurements, as part of lipid evaluation for atherosclerotic risk, are important as they provide the only convenient and cost-effective method for routinely estimating LDL cholesterol. Their inverse relationship to HDL makes triglyceride measurements a cost effective screening procedure. Unlike cholesterol measurements, triglycerides should always be assessed in the fasting state.

Phospholipids:

They are complex lipids resembling triglycerides, but in addition to glycerol and fatty acids, they contain one or more phosphoric acid groups and a nitrogenous base. The major phospholipids in plasma are lecithin and sphingomyelin. The phosphate and nitrogenous base are water soluble, a fact that is important in lipid transport. (Normal values range between 150-275 mgs %).

The Lipoproteins of Plasma:

Lipids are insoluble in water. When lipids combine with water soluble complex proteins, they become soluble and constitute lipoproteins.

The role of protein-lipid complexes in maintaining the lipids in solution in plasma was suspected by Schulz (1897) and Nerking (1901) at the turn of 20th century. Precipitation of a lipoprotein from horse serum was achieved in 1929 by Macheboeuf. Application of new physical methods for protein separation, including electrophoresis and ultra centrifugation expedited the progress in lipoprotein chemistry. Tiselius et al in 1941 reported the existence of two lipoprotein classes, separable by moving boundary electrophoresis. These were alpha and beta lipoproteins. It was another decade before a further component, Pre-B lipoprotein was identified by zonal electrophoresis (Dangerfield WO, 1955).

Lipoprotein	Electrophoretic Mobility	Major Apolipoprotein
Chylomicrons	Origin	Apo B – 48
Chylomicron remnants	Slow pre – ß	Apo B – 48
VLDL	Pre – ß	Apo B – 100
IDL	Slow pre – ß	Apo B – 100
LDL	ß	Apo B -100
HDL	A	Apo A - I
Lp(a)	Pre – ß	Apo B - 100

Table 3: Major Lipoprotein Classes

Structure of Lipoprotein Particle:

The lipoproteins are high molecular weight globular proteins that transport non polar lipids in the plasma. Each lipoprotein particle contains a non polar core, and a polar coat. The core consists of varying amounts o triglycerides and cholesterol esters forming bulk of the particle. The polar coat consists of phospholipids and apolipoprotein. The phospholipids stabilize the lipoprotein particles so that it can remain attached to unesterified cholesterol. The apolipoproteins are partly exposed on the surface so that it directs the lipoprotein to the site of its metabolism either by binding to specific enzymes or transport proteins on cell membranes.

Among the many techniques used to separate lipoproteins, the following are important.

1. **Ultracentrifugation:**

There are two principle ways of using ultracentrifugation to determine lipoproteins. They employ two different instruments. The preparative and the analytical centrifuge.

a) **Preparative ultracentrifugation**: Plasma has a salt density of about 1.006. Ultracentrifugation of plasma without adjustment of its density for a short period brings the chylomicrons rapidly to the top of the tube. Longer ultracentrifugation at this density allows the VLDL to be collected on the

surface. Addition of salt to plasma will further raise the density to selected levels and permit isolation of other lipoproteins. Those lipoproteins, which are separated below a density of 1.006 constitute VLDL, in the density range of 1.006 to 1.063 constitute LDL and in the density range of 1.063 to 1.21 constitute HDL.

b) **Analytical Centrifugation**: In this instrument, plasma fraction usually prepared at salt density of 1.063 is centrifuged at high speeds and the moving bands of the floating lipoproteins are serially photographed and later used to determine the concentrations that are referred to certain standard conditions.

2. **Electrophoresis:**

If the plasma is examined by paper or agarose gel electrophoresis at pH 8.6, it is possible to demonstrate, by means of fat stains, the existence of four bands. Three of these move towards the anode and one remains at the origin, that is, the line of application of the serum. The faster moving band occurs approximately in the same position as alpha-l globulin. It is known as alpha-lipoprotein and corresponds to the HDL fraction, demonstrated by ultracentrifugation floatation technique. The next band appears at approximately the position of beta-globulin and is known as beta lipoprotein, which corresponds to LDL fraction. Another band appears between the position of alpha globulin and beta globulin. It is known as pre-beta lipoprotein which corresponds to VLDL fraction. The band, which

remains stationary at the origin, consists of the chylomicrons. The five principal lipoprotein classes are defined according to their density on ultracentrifugation and by their mobility on agarose gel electrophoresis. In addition, they can be classified on the basis of size and relative concentrations of cholesterol or triglyceride and by their apoprotein content. The major lipoprotien classes are high-density lipoproteins (HDLs), intermediate-density lipoproteins (IDLs), low density lipoproteins (LDLs), very low-density lipoproteins (VLDLs), and chylomicrons.

Chylomicrons:

Chylomicrons are the largest of the lipoproteins. Their primary function is to transport dietary, or exogenous, triglycerides and cholesterol from the intestinal lumen to the sites of metabolism or storage. The chylomicrons are formed in the gastrointestinal (GI) tract. In the lumen of the GI tract, dietary fat is degraded into free fatty acids and monoglycerides. These substances enter the intestinal villi, where they are reconstructed into a triglyceride particle. Dietary cholesterol absorbed into the intestinal wall is then esterified to cholesteryl esters, mainly cholesteryl oleate, by the enzymatic reaction catalyzed by cholesterol acyl transferase. The triglyceride and cholesteryl esters are then combined with apos beta-48, A-I and A-IV within the intestinal wall to form chylomicron particles.

The nascent chylomicrons enter the systemic circulation by the way of lymphatics. Apos E and C are then added to the particles. Normally, the

chylomicrons are cleared rapidly from the blood and are virtually absent in the fasting state. The clearing of the chylomicrons is modulated by the enzyme lipoprotein lipase (LPL). Lipoprotein lipase catalyzes hydrolysis of the triglyceride core of the chylomicron, leaving a remnant particle rich in cholesterol, apo C, apo E, and apo B-48. During this process, apoproteins, phospholipids, and cholesterol from the surface of the chylomicron are transferred to HDL particles. The chylomicron remnants are cleared rapidly from the circulation by receptors present on the surface of liver cells. These receptors recognize the apo E component of the remnant particles. Remnants that contain the apo E2 moiety bind less well, and are thus removed less quickly than remnants that contain either the apo E3 or E4 moiety. Chylomicron remnants are thought to be atherogenic, and an abnormal delay in their clearance is therefore undesirable. Delay in chylomicron clearance may be secondary to a genetically inherited deficiency of LPL or its activator, apo C-II. It is partial degradation of the chylomicron to a remnant that renders the particle atherogenic. Delayed clearance of the remnant particles may damage the vascular endothelium, and thus predispose to atherosclerosis.

Hyperchylomicronemia also may be secondary to other acquired hypertriglyceridemic states, such as those seen with exogenous estrogen use, uncontrolled diabetes, and excessive alcohol intake. The presence of chylomicrons

in the serum is necessary for the diagnosis of type I or V hyperlipoproteinemia in the Fredrickson and less classification system.

Very Low-Density Lipoprotein:

VLDLS are intermediate in size between chylomicrons and IDLS. They are relatively large particles, with diameters ranging from 500 to 800 A. VLDLS are produced in the liver. Their primary lipid component is triglyceride, but cholesterol, cholesteryl ester, and phospholipid are also present. Their surface components are apo B-100, C and E and phospholipid. The synthesis of VLDL is increased by excess carbohydrate, alcohol, or caloric consumption. The function of VLDL is to transport endogenously synthesized cholesterol and triglycerides to the peripheral tissues, where the fatty acids can be utilized for energy or stored as triglycerides.When VLDL particles enter the systemic circulation, their triglyceride core is hydrolyzed by LPL. As the VLDL particle is degraded, most of its surface apoproteins, except for apo B-100, are transferred with other surface components to HDL. The remaining VLDL remnant is called IDL. Unlike the chylomicron remnant, IDL contains apo B-100 rather than apo B-48. The metabolism of VLDL is complex and not fully understood. Some of the larger particles appear to be directly removed from the circulation. The rest of the particles enter the cascade, in which they are converted to IDL and eventually to LDL.

Intermediate Density Lipoprotein:

IDLs, which carry both cholesterol and triglyceride, are the products of the enzymatic (LPL-medicated) breakdown of VLDL. After their formation, IDLs may be removed by the liver by means of the binding of apo E to the LDL or B/E receptor. The remainders are converted to LDL, a process, thought to be mediated, by hepatic triglyceride lipase. IDLs have high cholesterol content and migrate in the beta region on electrophoresis. Elevations of IDSs are thought to predispose to premature CAD and peripheral artery disease. Accumulation of IDL is characteristic of dysbetalipoproteinemia, also called Fredrickson's type III hyperlipoproteinemia. This relatively uncommon form of hyperlipoproteinemia is associated with both triglyceride and cholesterol elevations.

Low Density Lipoprotein:

LDL, which is 45 percent cholesterol by weight, is the major carrier of cholesterol to the nerve tissue, cell membranes and other cells that require the cholesterol for metabolic functions, including the synthesis of steroid hormones. LDLs have a density of 1.019 to 1.063 gm/ml a diameter of 180 to 280 A, and beta electrophoretic mobility. LDL usually is formed from VLDL breakdown. Direct synthesis has not been completely excluded. Increase LDL synthesis may occur be means of enhanced conversion of VLDL remnants or direct hepatic production of apo B containing lipoproteins.

Apo B-I00 is the only protein found in LDL, and makes up about 20 percent of the LDL mass. Each particle is thought to contain 1 molecule of apo B- 100, but the ratio of protein mass to total particle mass can vary from the large to the small particle range. LDL particles are heterogeneous, differing in their hydrated density and cholesteryl ester in the LDL particle, for example, may vary upto 40 percent by weight. Patients with greater concentration of small, dense LDL, have been reported to have a three times greater risk for acute myocardial infarction (MI), regardless of weight or gender. Small, dense LDL molecules are commonly associated with male gender, diabetes, depressed HDL levels and familial combined hyperlipoproteinemia.

LDL particles are recognized by specific LDL or apo B/E receptors on the surfaces of hepatic and certain non hepatic cells. These receptors also recognize and bind some of the apo E containing IDL particles, preventing their conversion into LDL. Bound LDL particles (and IDL particles) are then internalized into the cells. About 75 percent of the LDLs in the bloodstream are removed by this specific receptor mediated binding. The reminding LDL particles are cleared by scavenger or macrophage receptors or by non receptor mediated mechanism. The number of LDL receptors is not fixed, and can be modified by genetic defects, saturated fat and cholesterol intake or certain pharmacological agents.

The prototype disease involving the LDL receptor is familial hypercholesterolemia. In this condition, heterozygotes have a 50 percent reduction in LDL receptors, whereas homozygotes have little or no receptor activity. Familial hypercholesterolemia is fairly common, occurring in 1 in every 500 people. Familial combined hyperlipidemia (FCR) is even more common, possibly occurring in 1 in every 500 people. Clinically, FCR patients may be difficult to differentiate from those with familial hypercholesterolemia. In FCR, most patients lack tendon xanthomas; most family studies show varying Fredrickson's phenotypes.

The characteristic defect in FCR is thought to be an overproduction of apo B- 100 by the liver. Also, FCH patients have a lower ratio of apo A-I to apo B-100. About 80 percent of patients with an elevated LDL value do not have only one gene defect; the dyslipidemia is secondary to polygenic factors. Hence, elevations of LDL due to primary receptor defects are relatively uncommon.

Phenotype	Lipoprotein Abnormality	Result
Type I	Chylomicrons elevated	Very high TG
Type IIa	LDL elevated	High cholesterol
Type IIb	LDL and VLDL elevated	High cholesterol and TG
Type III	IDL elevated	High cholesterol and TG
Type IV	VLDL elevated	High TG, normal to slightly high cholesterol
Type V	Chylomicrons present and VLDL elevated	Very high TG and Cholesterol

Table 4: Fredrickson Classification of Hyperlipidemia

High Density Lipoprotein:

HDLs are produced by the liver and the GI tract and by the peripheral catabolism of chylomicrons and VLDLs. HDL particles carry cholesteryl ester as their major lipid and apos A-I and A-II as their major proteins. Much of the apoprotein component of HDL is transferred in the systemic circulation to VLDLs or chylomicrons. Apo C-II an obligatory activator of LPL, is one of the apoproteins transferred by HDL. By weight, HDL particles are about 30 percent cholesterol, 45 percent protein, and 25 percent phospholipid (predominantly phophatidyl choline). Small amounts of triglycerides are present.

HDL particles exist in several subtypes. For clinical purposes, HDL_2 and HDL_3 are the major circulating sub fractions. HDL_2, which migrates with alpha mobility, is the subfraction, most closely associated with statistical protections against premature atherosclerosis. It has a density of 1.061 to 1.201 gm/ml and a diameter of 90 to 120 A. HDL_3 is a smaller particle, with a density of 1.125 to 1.210 gm/ml and a diameter of 50 to 90 A. Alcohol consumption increases both HDL subfractions, with a greater impact on HDL_3 Lower levels of both subfractions are associated with male gender, hyper triglyceridemia, diabetes mellitus, obesity, uremia, the use of androgens, progestins, and tobacco products; and diets rich in polyunsaturated fats but low in total fat content.

Several epidemiological studies have addressed the debate whether there is a varying clinical impact on CAD depending on the relative levels of HDL_2 and $HDL_{3.}$ In males with CAD who have an associated low level of circulating HDL, both fractions of HDL are depressed with more of a decline in HDL_2.

HDL particles participate in the reverse transport of free cholesterol from peripheral tissues. Oram and co-workers report that apo A-I and A-II interact with this putative HDL receptor. These receptor mediated reverse cholesterol transports explain why persons with higher HDL concentrations are less prone to develop CAD.

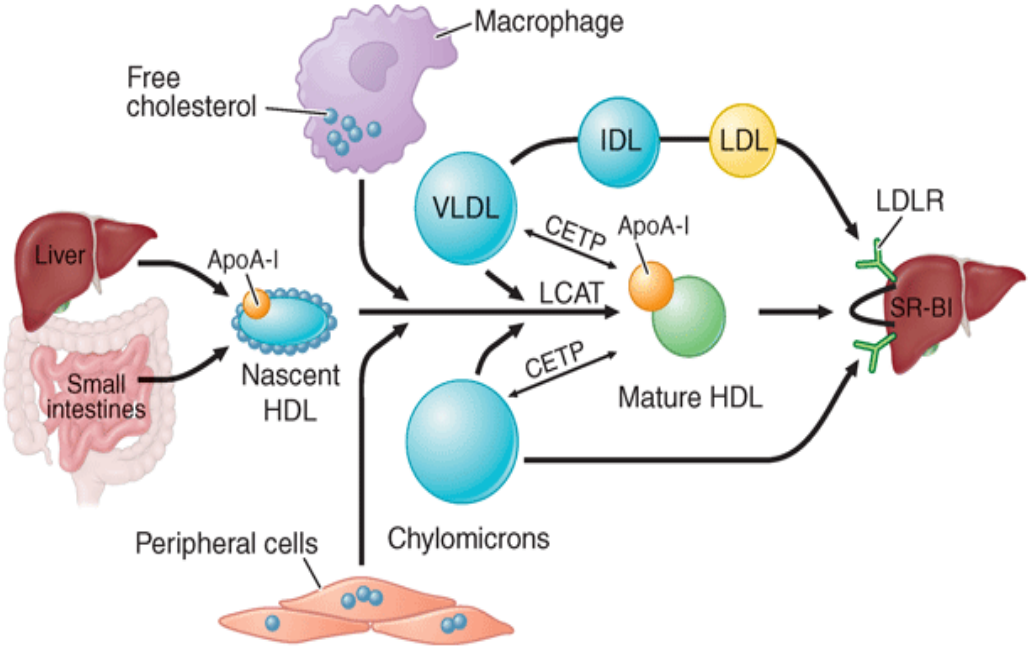

Fig. 6: Reverse Cholesterol Transport

Another explanation for the inverse relation between HDL levels and CAD incidence may be related to the observation that most patients with low levels of HDL have elevate levels of the more cholesterol and triglyceride rich lipoproteins. In this case, Low HDL levels may serve only as a marker for other, concurrent lipid abnormalities. Just as low levels of HDL are statistically associated with atherosclerosis, HDL is increased on a genetic basis in familial hyperapolipoproteinemia. The condition has been described as being associated with longevity.

Lipoprotein (A):

Lipoprotein (a), or Lp (a), has been established as an independent CAD risk factor. The structure of Lp (a) is similar to that of an LDL molecule linked by a

disulfide bridge to apoprotein (a). It has a density of l.085gm/ml and a size of 25A and it migrates in the prebeta region. Lp (a) levels range from l mg/dl to 100 mg/dl, with the largest number of values below 20mg/dl.

Although Lp (a) is structurally similar to LDL, the former appears to be regulated independently and carries an independent relation to overall coronary risk. If serum levels of both LDL and Lp (a) are elevated, the risk of CAD is markedly increased. Recent angiographic studies have documented a positive correlation between Lp (a) levels and the severity of coronary atherosclerosis.

The mechanism by which high levels of Lp (a) are related to coronary atherosclerosis is unclear. It has been suggested that because of the structural similarities of Lp (a) to plasminogen, high levels of Lp (a) may inhibit the thrombolytic activity of naturally occurring tissue plasminogen activity. Plasminogen is composed of five sequences of amino acids rich in cysteine. Each sequence is called a kringel. Lp (a) lacks the first three kringles, but has a sequence that is highly homologous to the fourth kringle of plasminogen. This particular sequence is repeated 37 times in the Lp (a) molecule. There is no serine protease activity in Lp (a) and no thrombolytic activity. An alternate explanation for the association between elevated Lp (a) levels and atherosclerosis is that Lp (a) may somehow alter the LDL mediated delivery of cholesterol to the atherosclerotic plaque.

The control mechanisms of Lp (a) are unknown. Dietary changes that increase LDL levels do not affect Lp (a) levels. The effects of pharmacological agents are unclear, although Lp (a) has been reported to be decreased by niacin, neomycin, and stanozolol.

Secondary Causes of Hyperlipidemia:

LDL	
Elevated	**Reduced**
Hypothyroidism Nephrotic syndrome Acute intermittent porphyria Cholestasis Anorexia nervosa Hepatoma Drugs Thiazides, carbamazepine, cyclosporin	Severe liver disease Malnutrition Malabsorbtion Gaucher’s disease Chronic infectious disease Hyperthyroidism Niacin toxicity

HDL	
Elevated	**Reduced**
Alcohol Exercise Estrogens Exposure to chlorinated hydrocarbons	Smoking Type 2 DM Obesity Malnutrition Anabolic steroids, beta blockers Gaucher's disease

VLDL elevated	IDL elevated
Type 2 DM Obesity Alcohol Hepatitis Glycogen storage disorders Renal failure Sepsis Stress Cushing's syndrome	Hypothyroidism Multiple myeloma Monoclonal gammopathy Autoimmune disease

Pregnancy Acromegaly Lipodystrophy Estrogen, beta blockers, glucocorticoids, bile acid binding resins, retinoic acid	

Chylomicrons Elevated	**Lp(A) Elevated**
Autoimmune disease Type 2 DM	Inflammation Renal insufficiency Menopause Orchidectomy Hypothyroidism Acromegaly Nephrosis Growth hormone, isotretinoin

Table 5: Secondary Causes of Hyperlipidemia

Lipids and Atherosclerosis

It is well established that hypercholesterolemia due to elevated blood levels of LDL is a major cause of CHD and that lowering elevated levels of cholesterol will reduce the risk of coronary disease. Inverse relationship is noted with HDL. There is also a weak correlation between plasma triglycerides and the incidence of coronary artery disease. The exact mechanism of atherogenesis is still in controversy. LDL particles which become oxidized (by natural process) may be particularly atherogenic. Receptors on the surface of macrophages within the plaque, binds and accumulates oxidized LDL. The formation of antibodies to oxidized LDL is also important in plaque formation. There is a possibility that chronic hyperlipidemia may initiate endothelial injury which stimulates atherosclerosis. This injury favours adherence of monocytes and lymphocytes at the focus of injury, in part owing to the stimulation of endothelial cell synthesis of adhesion molecules. It induces a change in the platelet membrane composition leading to activation and increased adhesiveness of platelets. With chronic hyperlipidemia, lipoproteins accumulate within the intima at sites of endothelial injury or dysfunction.

A strong negative correlation has been demonstrated between plasma levels of HDL cholesterol and CHD. Most of the variability in HDL cholesterol levels

reflect HDL_2 levels; plasma levels of the other major HDL subclass, HDL_3, are fairly constant both intra and inter-individually.

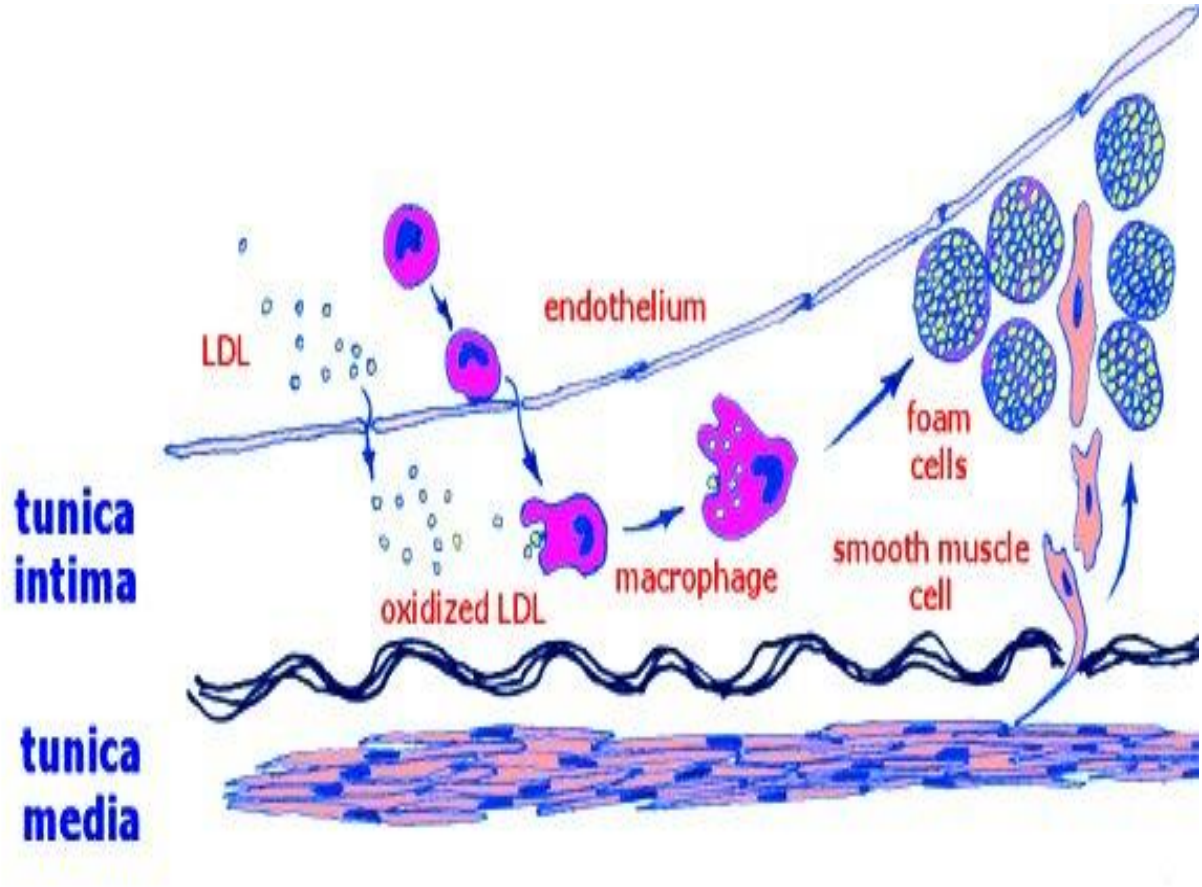

Fig. 7:Pathogenesis of Atherosclerosis

HDL particles secreted into the circulation by liver and cholesteryl ester enriched macrophages are complexes of apolipoproteins and phospholipids and acquire unesterified cholesterol originating in cell membranes during cell renewal or death. Their major phospholipid is phosphatidylcholine or lecithin.

Both lecithin and unesterified cholesterol serve as substrates for the cholesterol-esterifying enzyme, lecithin cholesterol acyltransferase (LCAT), which circulates with HDL in the plasma. LCAT acts on the nascent HDL particles to generate a core of cholesteryl esters and to change the structural transition to mature, spherical HDL particles. LCAT also acts on mature HDL_3 particles, i.e., after they have acquired cholesterol and lecithin not only from cell membranes but also from chylomicrons and VLDLs during those particles lipolysis. Generation of cholesteryl esters by the LCAT reaction leads to enlargement of the small HDL_3 particles such that they are converted into the larger HDL_2 particles. Formation of HDL_2 increases the cholesterol-carrying capacity of HDL, and HDL cholesterol levels raise This drives the process termed "reverse cholesterol transport", in which HDL returns cholesterol from peripheral tissues to the liver for excretion into the bile.

The cholesteryl esters of HDL, particularly of HDL_2 formed de novo by the LCAT action, need not remain within the HDL core. They can be transferred from HDL to the triglyceride rich lipoproteins, i.e., chylomicrons and VLDL in exchange for triglyceride molecules. This heteroexchange of insoluble cholesteryl esters and triglycerides between HDL and triglyceride rich lipoproteins is catalyzed by the action of cholesteryl ester transfer protein. The transferred

triglycerides are hydrolyzed from the HDL core by hepatic lipase, located in the endothelial cells of the liver. Only cholesteryl esters remain in the core those that were not exchanged for triglycerides. Hence HDL_2 particles are converted back into the smaller HDL_3 particles. This mechanism is the basis for the well established clinical observation that, individuals with permanent or temporary hypertriglyceridemia (due to increased levels of VLDL or due to accumulation of chylomicrons in the course of postprandial lipemia) have low HDL_2 and low HDL cholesterol levels.

The cholesteryl esters transferred from HDL and the triglyceride rich lipoproteins remain with the latter particles along their lipolytic cascades and the endocytotic pathways of their remnants (i.e., the LDL receptor and the scavenger pathways). Thus, transfer of cholesteryl esters from HDL to triglyceride rich lipoproteins may contribute to the atherogenic potential of chylomicrons and VLDL, in that “good” cholesterol is turned into “bad” cholesterol.

Although there is no clinical trial directly demonstrating the benefit of increasing HDL cholesterol alone, Helsinki Heart Study results suggests that increasing HDL add benefit to lowering LDL in CHD risk reduction. Similarly, data from the Prospective Cardiovascular Muenster (PROCAM) Study have shown that the combination of LDL elevation, hypertriglyceridemia, and low HDL confers greater risk of CHD than elevated LDL alone. There are several causes for

low serum HDL cholesterol levels. Heavy cigarette smoking is a documented cause. Obesity is an apparent association, as is a sedentary lifestyle. Hypertriglyceridemia is frequently associated with very low HDL levels. Certain drugs can also have an appreciable depressing effect on HDL. Finally, there is evidence that isolated low HDL cholesterol, termed hypoalphalipoproteinemia, may be genetically transmitted in an autosomal dominant fashion. Patients with this disorder have a normal lipid profile other than the low HDL but an apparently increased risk for atherosclerosis.

The recommended methods for improving HDL cholesterol values are non-pharmacologic; smoking cessation, weight reduction, regular and vigorous exercise and alteration of offending drugs, if possible. As yet, there is no direct evidence that drug-induced increases of low HDL cholesterol in the setting of normal LDL cholesterol and triglyceride levels are beneficial in CHD risk reduction. HDL cholesterol levels can be increased with gemfibrozil (the agent used in the Helsinki study) or nicotinic acid.

The possibility that chronic hyperlipidemia, and particularly hypercholesterolemia, may itself initiate endothelial injury has enticed many investigators. Much evidence suggests that hypercholesterolemia has a variety of adverse effects.

It increases the cholesterol- phospholipid ratio of endothelial cell membranes, rendering them more rigid and less able to maintain their normal intercellular associations, potentially increasing permeability and in effect causing a subtle form of endothelial injury.

It favours the adherence of monocytes and lymphocytes to the focus of injury, in part owing to the stimulation of endothelial cell synthesis of adhesion molecules.

It induces changes in platelet membrane composition, leading to activation and increased adhesiveness of platelets.

With chronic hyperlipidemia, lipoproteins accumulate within the intima at the site of endothelial injury or dysfunction. Although significant amounts may enter the arterial wall, there is some concurrent efflux, perhaps mediated by HDL. Most important, it provides the opportunity for oxidation of lipoproteins, yielding modified LDL.

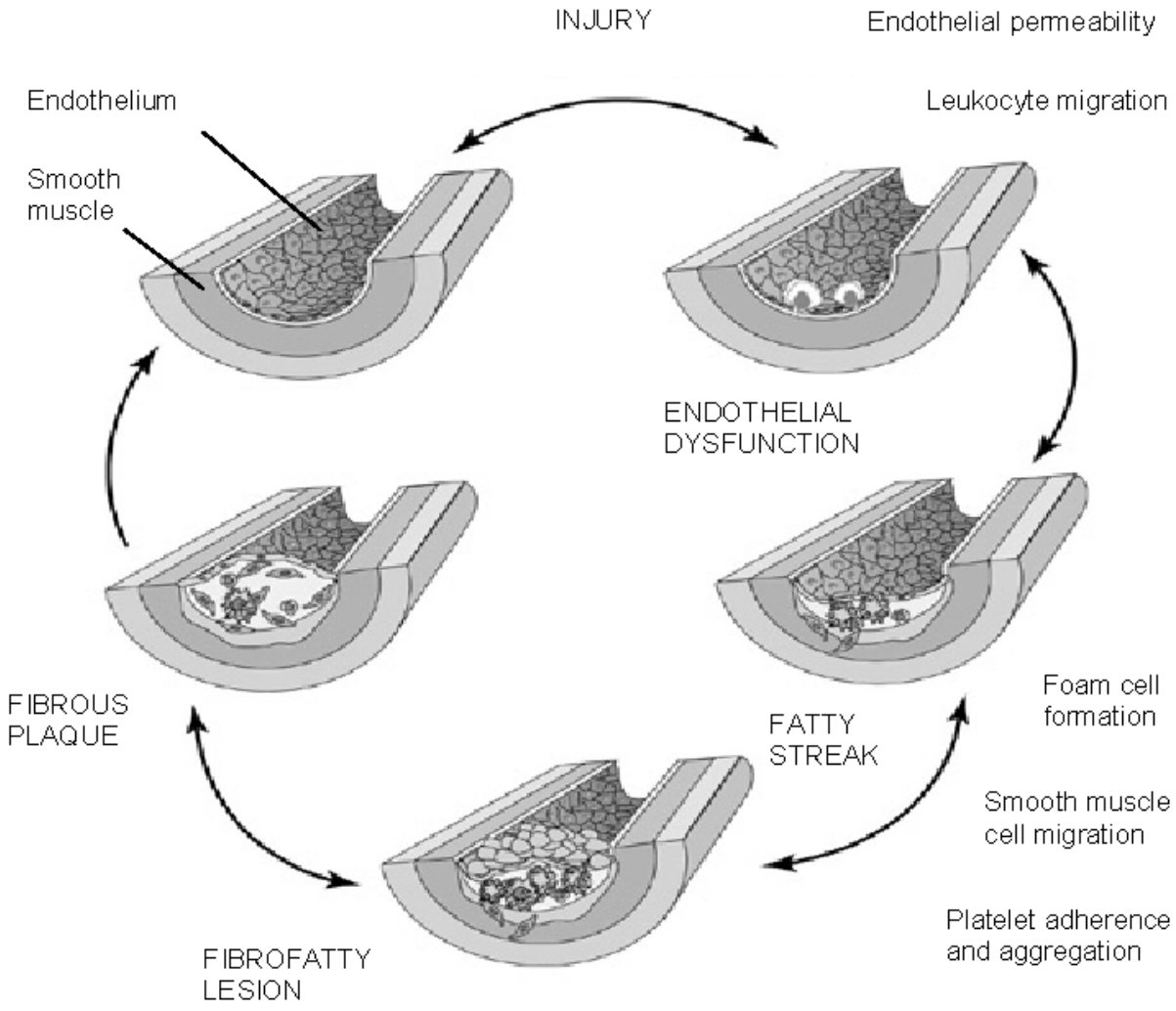

Fig. 8: Pathogenesis of Atherogenesis

Apoproteins:

Apoproteins are key lipoprotein components that serve both as enzymatic co-factors and as recognition elements that bind to specific receptors on peripheral tissues, including the vascular endothelial cells. It is the apo E component of the chylomicron remnant, for example, that is recognized by receptors on the

hepatocyte. The apoproteins are distinguished alphabetically and numerically as apo A-I through apo E.

A great deal of research has been conducted in the use of apoproteins as CAD markers. Some investigators have found that the concentration of apo A-I and apo B-100 are better predictors of CAD than are measurements of total plasma lipids or lipoproteins. In one study, apo A-I was the best predictor of atherosclerotic risk in patients undergoing coronary arteriography. In this study, higher levels of apo A-I were associated with a deceased prevalence of obstructive coronary lesions. Indeed, apo A-I was found to be a better CAD predictor than either total cholesterol or HDL.

Apoprotein A:

Apo A-I, the prototype of apo A, is a major protein in HDL and also is seen in chylomicrons. It has a molecular weight of 28,000 and is synthesized in the GI tract and liver. Its specific regions called amphipathic helices are enriched with charged amino acids that form areas of polar and non-polar residues. Apo A-I functions as the activator of lecithin cholesterol acyl transferase (LCAT), and also has been found as a degradation product in amyloid fibrils.

Apo A-II is a minor constituent of HDL and does not appear to be present in all species. Human apo A-II is of hepatic origin and consists of two identical chains attached by a single disulfide linkage. Apo A-II may be an activator triglyceride and phospholipid while utilizing HDL_2 as its preferred substrate. Apo A-IV is synthesized in the gut and is present in HDL, chylomicrons and as a free protein. Its molecular weight is 46,000, and its structure is helical. Although its function is not known, it also may be an activator of LCAT.

The genetic codes for apos A-I, A-IV and C-III are close together on the long arm of chromosome 11. Combined A-I/C-III deficiency is associated with severe premature atherosclerosis.

Apoprotein B:

Apo B occurs in two forms. Apo B-48 is synthesized by the small intestine, and apo B-100 is secreted by the liver. Apo B-48 is present on the surface of chylomicrons and chylomicron remnants. Apo B-100 is found in VLDL, IDL and LDL. Apo B-I00 is the primary apoprotein of LDL and accounts for 25 percent of its weight. It also is the recognition site for the LDL or apo B/E receptor on cell surfaces. It has recently been determined that a single gene regulates the synthesis of both apo B-48 and apo B-100. The gene for apo B-I00 has been localized to chromosome 2 and exists as a 40 kilobase structure. The structure in the amino acid sequence of human apo B-100 and the corresponding cDNA messenger have

recently been determined. A unique editing mechanism introduces a stop codon into the mRNA for apo B by means of a single base change. This allows the biosynthesis of two proteins from a single gene and mRNA, with either apo B-1 00 or apo B-48 being synthesized.

Apoprotein E:

Most apo E is synthesized in the liver. However, other tissues, including the small bowel, kidney, adrenals, and the cells of the reticuloendothelial system, have the ability to synthesize this apoprotein. Apo E accounts for about 15 percent of the protein content of VLDL, 7 percent of the protein content of chylomicron remnants and 2 percent of the protein content of HDL. It can be recognized by the LDL or apo B/E receptor and by specific apo E receptors in the liver whose function appears to be the removal of chylomicron remnants. Apo E is polymorphic and contains three major alleles; apos E2, E3, and E4. These respective alleles are present in about 10 percent, 76 percent, and 13 percent of whites. Their various combinations result in homozygtes for apos E2/2, E4/4. Also, apos E2/3, E2/4 and E3/4 exist in the heterozygous state. The polymorphism of apo E has been determined on a molecular basis and results from the substitution of an amino acid at residues 112 and 158 in the protein. About 90 percent of the patients with type III hyperlipoproteinema (HLP) are homozygous for the E2/2 phenotype. This disorder is characterized by hypercholesterolemia, hypertriglyceridemia and IDL

or VLDL particles abnormally enriched in cholesterol. These particles have beta electrophoretic mobility and are termed beta-VLDLs. Premature coronary and peripheral vascular disease is characteristically associated with type III HLP. Type III HLP is also by the delayed clearance of chylomicron remnants in the serum due to impaired binding of these remnants to the lipoprotein receptors in isolated cells. Because the E2/2 genotype occurs in 1 percent of the population, and type III HLP is rare, a second abnormality must be present.

Apo E isoforms may account for as much as 15 percent of the variability of cholesterol and LDL levels in the population. Also, recent Finnish studies suggest that E4 may be associated with increased cholesterol absorption in the GI tract. In the Prospective Cardiovascular Muenster (PROCAM) study, E2 was associated with lower cholesterol levels and E3 or E4 with higher levels of total cholesterol and LDL, in populations with and without CAD

Summary of Apoproteins:

Name	Lipoprotein	Function
Apo A-I	HDL.,Chylomicrons	Structural; activator of LCAT enzyme
Apo A-II	HDL.,Chylomicrons	Structural
Apo A-IV	HDL.,Chylomicrons, VLDL	Unknown
Apo B-IOO	LDL, VLDL	Structural; Synthesis and secretions of VLDL; bind to LDL receptor (B/E)
Apo B-48	Chylomicrons	Structural; synthesis and secretions from intestine
Apo C-I	HDL.,Chylomicrons,VLDL	Activator of LCAT
Apo C-II	HDL.,Chylomicrons,VLDL	Activator of lipoprotein lipase
Apo C-III	HDL.,Chylomicrons,VLDL	Stabilizes surface; provides negative charge
Apo D	HDL.,Chylomicrons	Cholesteryl ester exchange
Apo E	HDL.VLDL, Chylomicrons	Binds to receptor on cell membrane of liver (E and B/E) and macrophage

Table 6: Summary of Apoproteins

MANAGEMENT OF HYPERLIPIDEMIA:

Goals of Lipid Lowering Therapy:

Elevated LDL cholesterol: Treatment of elevated LDL cholesterol can have either of two aims.

Primary prevention of the complications of atherosclerosis or secondary treatment after complications has occurred. The rationale for primary prevention is based on the large body of data linking elevated levels of LDL cholesterol with increased CHD risk and an impressive body of clinical and experimental data demonstrating that reducing LDL cholesterol slows progression and may actually induce regression of atherosclerotic lesions. Both primary and secondary intervention trial indicate that total mortality can be reduced when the LDL Cholesterol is lowered:

A meta-analysis of four randomized trials28, 294S, CARE, AFCAPS/ Tex CAPS, LIPID) 30comparing HMG – COA reductase inhibitors to control included 30817 participants and found that HMG-COA reductase inhibitor treatment was associated with.

- 20% decrease in total cholesterol, and 5% increase in HDL cholesterol
- 28% decrease in LDL cholesterol, 13% decrease in triglycerides.
- 31% decrease in major coronary events and a 21% decrease in all cause mortality.

- Similar risk reduction in women and men
- Unexpectedly the risk of stroke was also reduced by 19 to 32% by HMG-COA reductase inhibitor treatment.

Primary Prevention:

The NCEP adult treatment panel III recommends measuring plasma cholesterol in all adults older than 20 at least every 5 yrs, with a fasting lipid panel i.e.TC, LDL-C, HDL-C and TG. If non-fasting lipid panel is obtained and the TG level is 200 mg/dl or HDL –C is less than 40 mg/dl, a follow up fasting lipid panel is recommended. Primary prevention goals include LDL cholesterol < 130mg/dl, Triglycerides <150 mg/dl and HDL cholesterol > 40 mg/dl for men and > 50 mg/dl for women.

Determination of Risk:

The patient's risk of future events is based on presence of known CAD or clinical atherosclerosis in a non-coronary bed, Diabetes mellitus (CAD equivalent) and other risk factors. These include age (men 45 years or older and women 55 yrs or older), smoking, hypertension, family history of premature CAD (defined as CAD in first degree male relatives before age of 55 years, and in a first degree female relative before age of 65 yrs) and low HDL < 40 mg/dl.

In individuals with zero or one risk factor, life style modification alone and follow up testing may be used if LDL < 160mg/dl. If LDL is > 140 mg/dl, drug treatment is indicated.

Ten-year risk of 10% to 20% target LDL –C for this group is less than 130 mg/dl and these patients should be initially approached with therapeutic life style change (TLC). If LDL –C is more than 160 mg/dl, consideration may be given to starting drug therapy.

CHD risk equivalents NCEP III recommend therapy with statins and TLCs if LDLC is 130 mg /dl are greater. In patients with LDL –C levels between 100 and 130 mg/dl. TLC with are without statins. HPS trial argued that (heart protection study) all patients with CHD should be treated with statins and TLC regardless of the LDL – C level.

Elevated Triglycerides:

The evidence that treatment to reduce plasma triglyceride levels or increase levels of HDL cholesterol leads to long term heath benefits is less compelling than that for treatment of high LDL levels.

Beneficial effects of niacin have been attributed, in part to its HDL raising effect and its action to reduce triglyceride and LDL. The management of hypertriglyceridemia focuses on the associated LDL and HDL concentrations as guidelines for therapy. Thus, the overall risk profile can be used to set goals for

LDL cholesterol using a low HDL level (commonly associated with hypertriglyceridemia) as a concomitant major risk factor for atherosclerosis.

However, when triglycerides levels are > 500 mg/dl, the risk of developing pancreatitis increase and a direct focus on lowering triglyceride is recommended Thus,TG levels > 500 mg/dl are generally treated with drugs where as lower levels (200 to 500mg/dl) are not treated unless other CHD risk factors are present.

Treatment Modalities:

- Therapeutic life style changes
- Dietary modifications
- Weight loss and exercise.
- Pharmacological treatment

Major Drugs Used for the Treatment of Dyslipidemia:

Drug	Major indications	Mechanism	Common side effects
HMG-COA reductase inhibitors(statins)	Elevated LDL	↓cholesterol synthesis ↓ hepatic LDL receptors ↓VLDL production	Myalgia, arthralgia, ↑ transaminases, dyspepsia
Bile acid sequestrants	Elevated LDL	↑ bile acid excretion ↑ LDL receptors	Bloating,constipation, ↑ TGL
Nicotinic acid	Elevated LDL, low HDL, elevated TG	↓ VLDL hepatic synthesis	Cutaneous flushing, GI upset, elevated glucose and uric acid, hepatitis maculopathy
Fibric acid derivatives	Elevated TG, Elevated remnants	↑ LPL, ↓ VLDL synthesis	Dyspepsia,myalgia,gall stones, ↑ transaminases
Fish oils	Severely elevated TG	↓ chylomicron and VLDL production	Dyspepsia, diarrhea
Cholesterol absorption inhibitors Ezetimibe	Elevated LDL	↓ intestinal cholesterol absorption	↑ transaminases

Table 7: Major Drugs Used for the Treatment of Dyslipidemia

MATERIALS AND METHODS

The study population includes 100 treatment naïve HIV patients, who are non-diabetic, non-obese and normotensive. The study was done in Government Rajaji Hospital, ART centre, Madurai during December 2013 to July 2014. HIV seropositivity was determined as per NACO guidelines.100 HIV negative age & sex matched controls were also included in the study. The ethics clearance was obtained from the Institutional ethical committee.

Inclusion Criteria:

- Age 20 to 40 yrs
- Newly diagnosed HIV positive patients who are willing to participate in the study.

Exclusion Criteria:

- Diabetes
- Hypertension
- Obesity (BMI > 25)
- Hypothyroidism
- Persons who are already on lipid lowering therapy

A detailed clinical profile including elaborate history, general examination and systemic examination was done for all patients included in the study. Appropriate investigations were carried out.

Blood Sampling and Laboratory Evaluation:

The blood samples for analysis after 12 hours of complete fasting. The subjects were asked to have their light, fat free diet on the day prior to sampling.

The Venepuncture was done in the cubital fossa. Tourniquet was used but was released just before sampling to avoid artifactural increase in the concentration of serum lipids.

About 10 ml of venous blood was drawn using perfectly dry and sterile disposable syringes. The serum was separated within 2 hours of collection to prevent artifactural changes in concentration of HDL and the samples were analyzed on the same day.

The lipid assay was done using the Dr.Lange LP 700 equipment.

Dr.Lange LP 700 laboratory system:

The lab system LP 700 consists of following instruments:

- LQV 018 suction device
- LTV 015 cuvette changer (Rack) thermostat

- LQV 016 Universal thermostat
- LP 700 photometer

The filters of the wavelength 578 nm, 548 nm, 492 nm, 405 nm, 340 nm belong to the LP 700 and are automatically recognized by it.

The following standard wavelengths 800 nm, 623 nm and 520 nm are available additionally.

The cuvette compartment accepts any 10 mm square cuvette as well as the Dr.Lange cuvette test.

The minimum volumes are

- The prefixed filling volume of Dr.Lange cuvette test.
- 450 microlitre for SM suction cuvette and
- 500 microlitre for SM square cuvette.

The methods designed for estimation of various lipid fractions are as follows.

Serum Cholesterol Estimation:

This was done based on CHOD-PAP method, the enzymatic calorimetric method.

Test Principle:

Cholesterol ester is hydrolysed by cholesterol esterase. Then free cholesterol is further oxidized to choles-4-En-3-one and hydrogen peroxide by the cholesterol oxidase. The formed hydrogen peroxide reacts with 4-amino antipyrine and phenol in the presence of peroxidase to produce pink coloured dye

Serum Triglyceride Estimation:

Triglyceride concentration was determined by enzymatic calorimetric test as per details shown in the system specific working instructions supplied with kits.

Method:

Enzymatic hydrolysis of triglycerides with subsequent determination of liberated glycerol by calorimetry.

Test Principle:

Triglycerides are hydrolyzed to glycerol and free fatty acids by lipases. Glycerol is phosphorylated by ATP in presence of glycerol kinase to glycerol 3-phosphate which is oxidized by the glycerol 3-phosphate oxidase releasing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-aminoantipyrine/3,5-dichloro 2-hydroxybenzene sulfuric acid to give a red color which is read at 510nm.

HDL Cholesterol Estimation:

This involves two steps- precipitation and cholesterol estimation of the HDL fraction by a modification of the method described by Burstein et al.

Test Principle:

Chylomicrons, VLDL, and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL in the supernatant; their cholesterol content is determined enzymatically.

In patients with high triglyceride values, the HDL cholesterol estimation was done after dilution of serum with isotonic saline in 1:1 ratio and the resultant value was multiplied by 2 to obtain HDL cholesterol. This was done to prevent the erroneous values of HDL-cholesterol due to impaired sedimentation of precipitate in serum with high triglyceride concentration.

LDL Cholesterol Estimation:

LDL cholesterol was calculated by using a standard WHO approved formula.

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL}$$

Following values were taken as normal

Total cholesterol <200 mgs/dl

Triglyceride<160 mgs/dl

HDL-C 40-55 mgs/dl

LDL-C <100mgs/dl

VLDL-C <35mgs/dl

The CD4+ T lymphocyte count was estimated by fluorescence activated cell sorter count system (Becton Dickinson).

The diagnosis of dyslipidemia was made as per National Cholesterol Education Program Adult Treatment Panel III.

LDL Cholesterol (mg/dl)	
<100	Optimal
100 – 129	Near/Above optimal
130 – 159	Borderline high
160 – 189	High
≥ 190	Very high

Total Cholesterol (mg/dl)	
< 200	Desirable
200 – 239	Borderline high
≥ 240	High
HDL Cholesterol (mg/dl)	
< 40	Low
≥ 60	High
Triglycerides (mg/dl)	
< 150	Normal
150 -199	Borderline High
200 – 499	High
≥ 500	Very High

Table 8: NCEP ATP III Guidelines

Outcome Measurement:

The results were analyzed by calculating averages, standard deviation. Student's unpaired to test was applied for comparison of study groups and P values were obtained. A P value of < 0.05 was considered statistically significant.

Results:

The mean age of patients was 33±4 years and of controls was 33±5 year. Ratio of male to female was 75:25 in cases and 80:20 in controls.

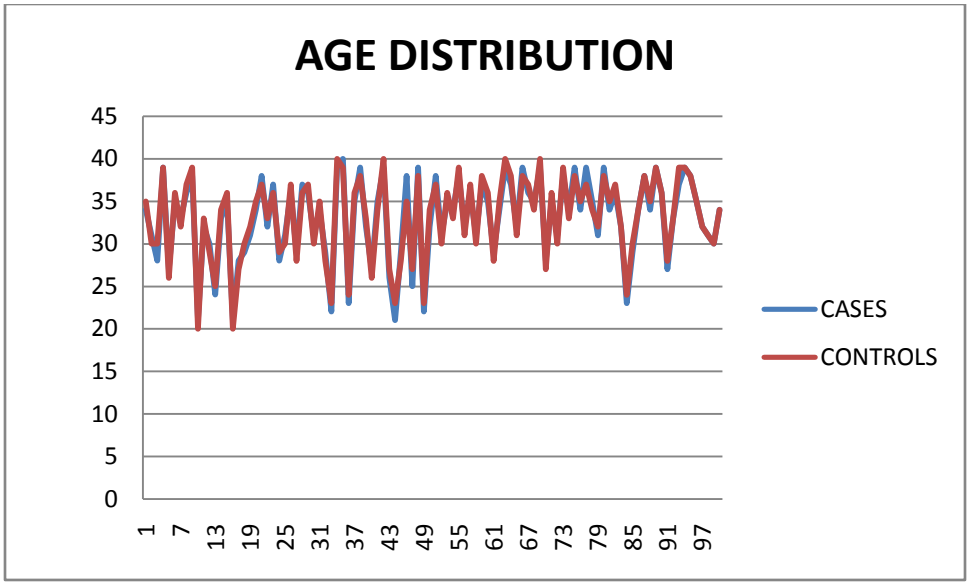

Fig. 9: Age Distribution

This graph shows almost equal distribution of age among both study groups .

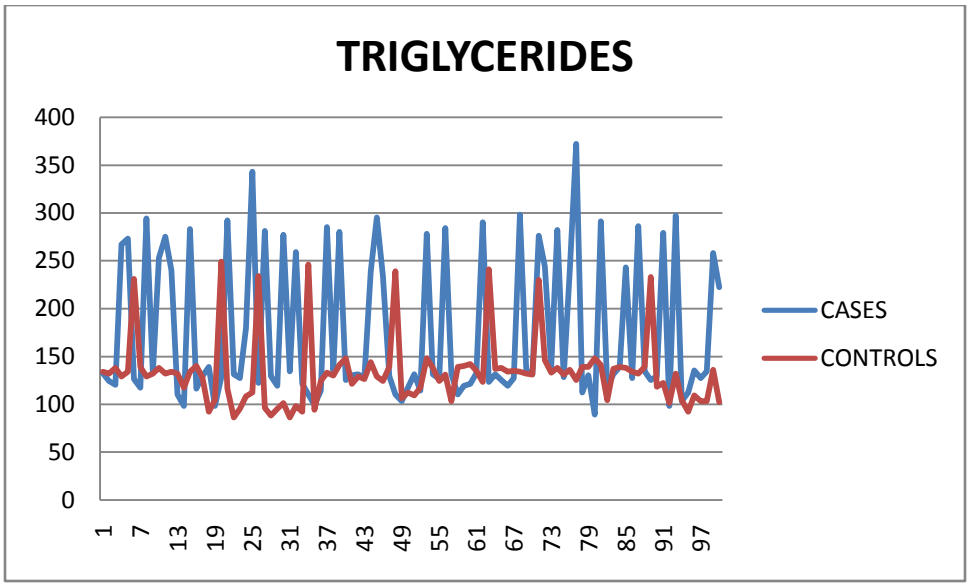

Fig. 10

Figure 10 showed the distribution of serum triglycerides (mg/dl) in both study groups and the mean values are 174±75 in cases and 133±35 in controls.

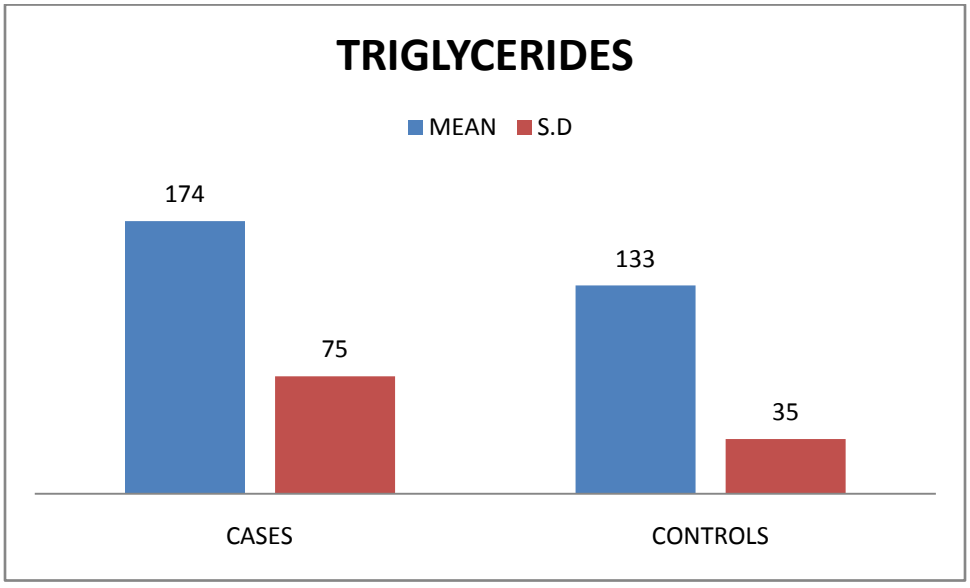

Fig. 11

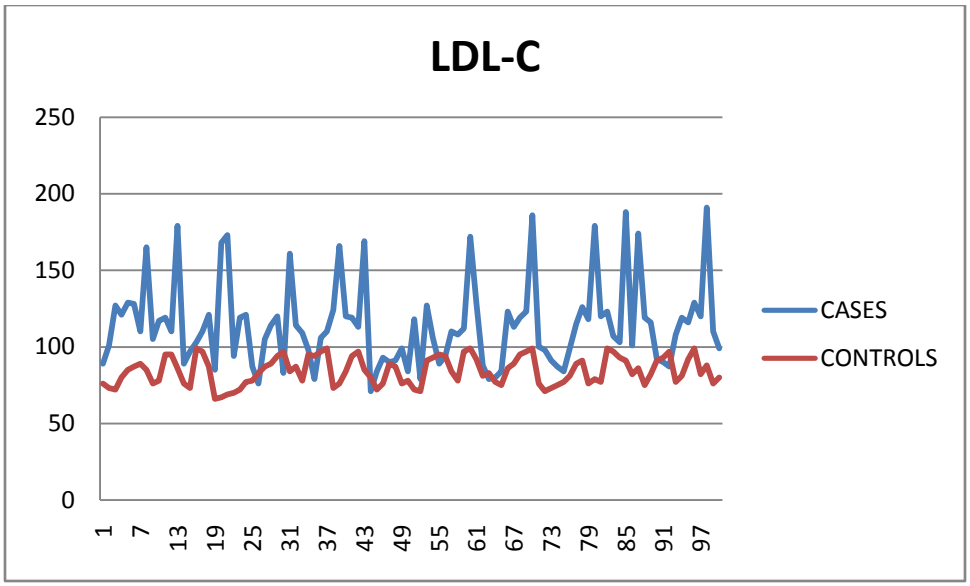

Fig. 12

Figure 12 showed the distribution of serum LDL-C (mg/dl) in both study groups and the mean values are 114±28 in cases and 84±9 in controls.

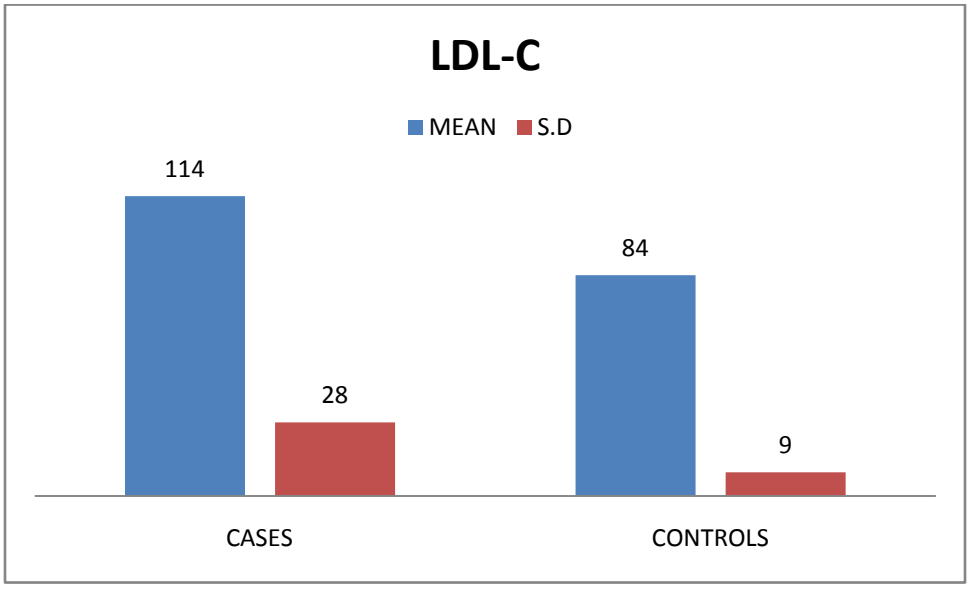

Fig. 13

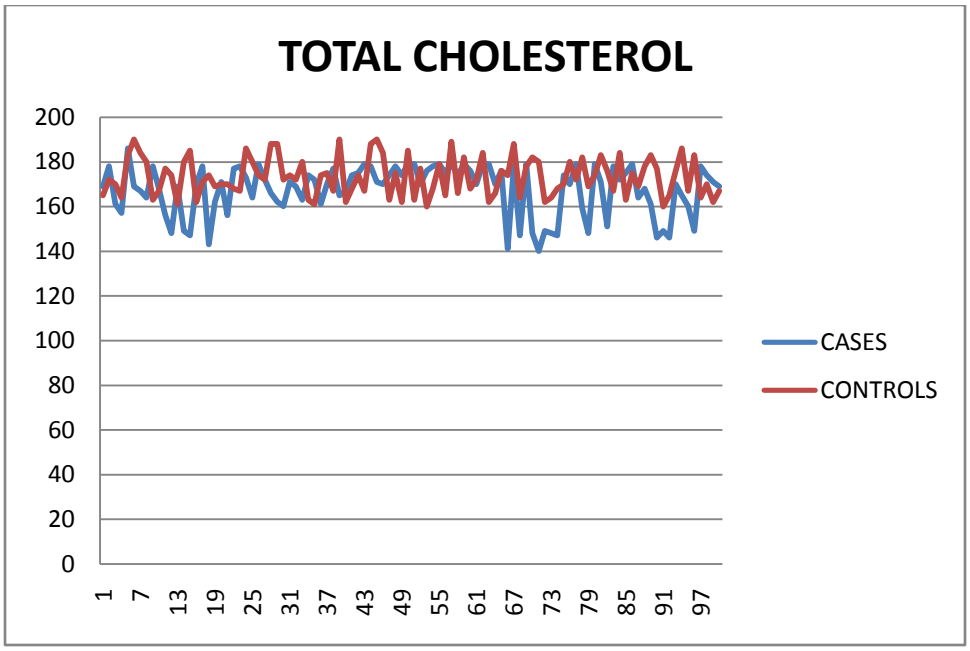

Fig. 14

Figure 14 showed the distribution of serum total cholesterol (mg/dl) in both study groups and the mean values are 167±11 in cases and 183±8 in controls.

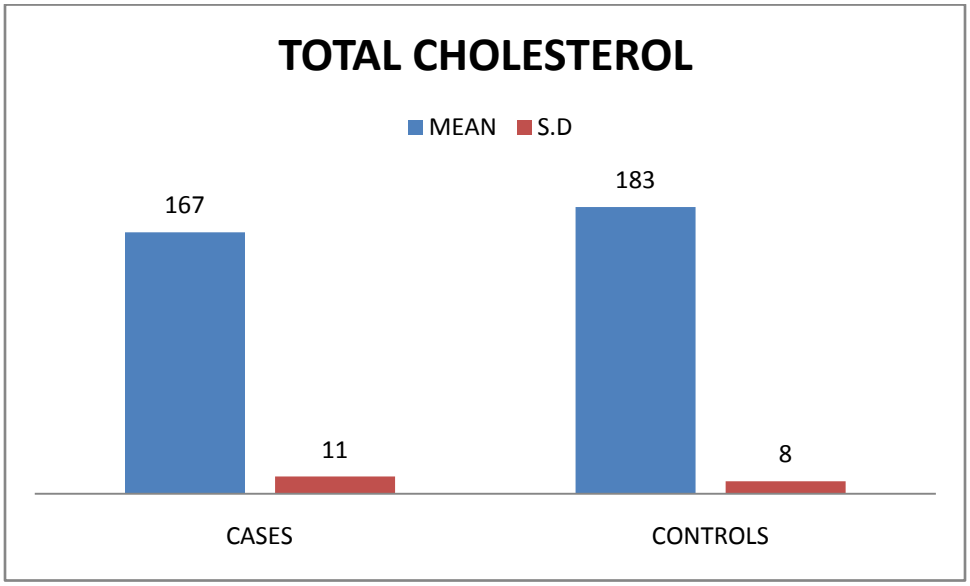

Fig. 15

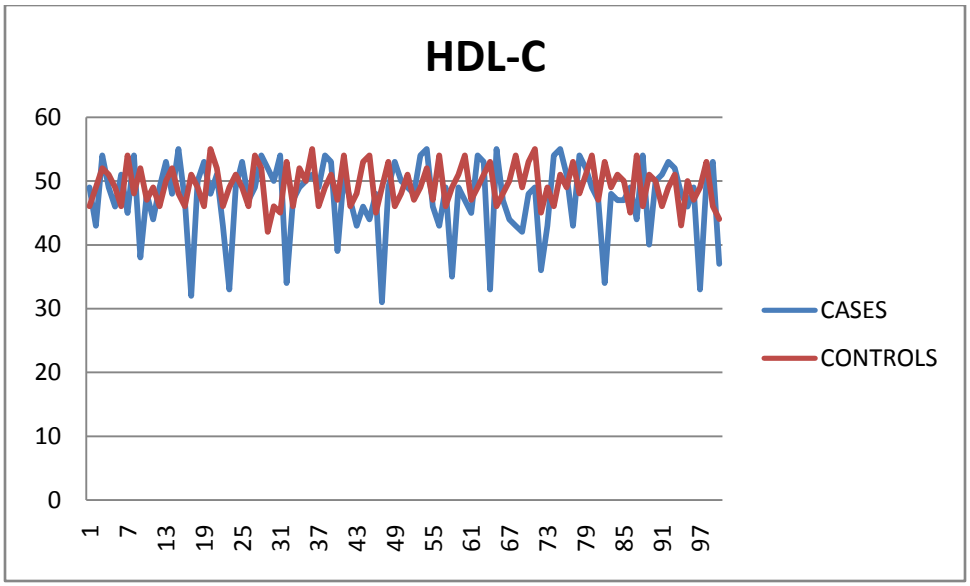

Fig. 16

Figure 16 showed the distribution of serum HDL-C (mg/dl) in both study groups and the mean values are 47±6 in cases and 49±3 in controls.

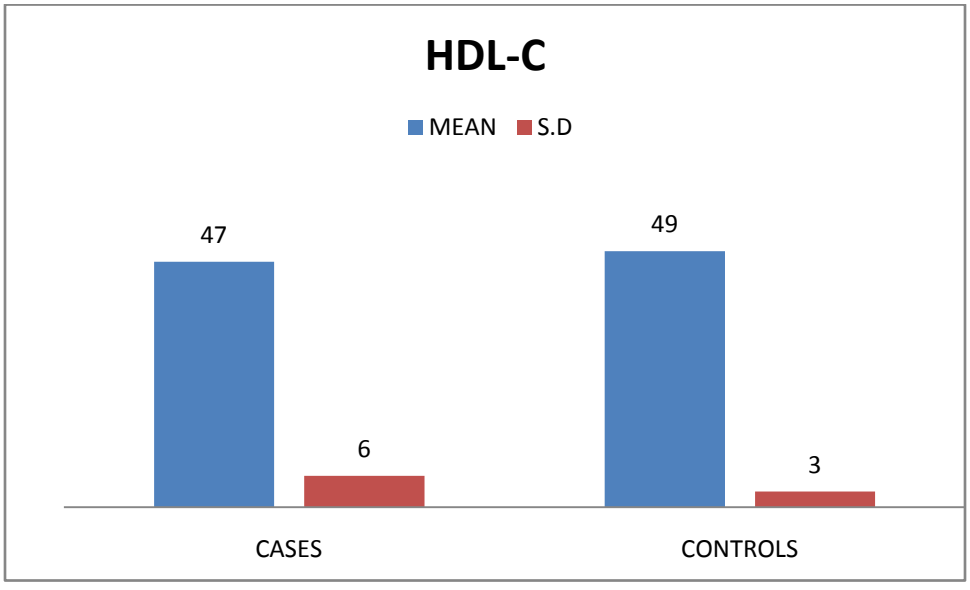

Fig. 17

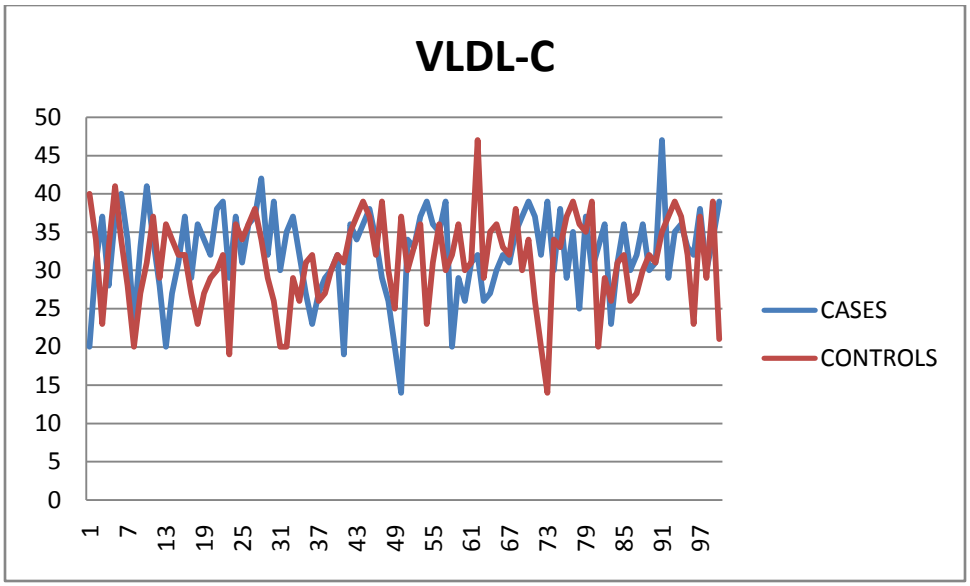

Fig. 18

Figure 18 showed the distribution of serum VLDL-C (mg/dl) in both study groups and the mean values are 32±6 in cases and 31±6 in controls.

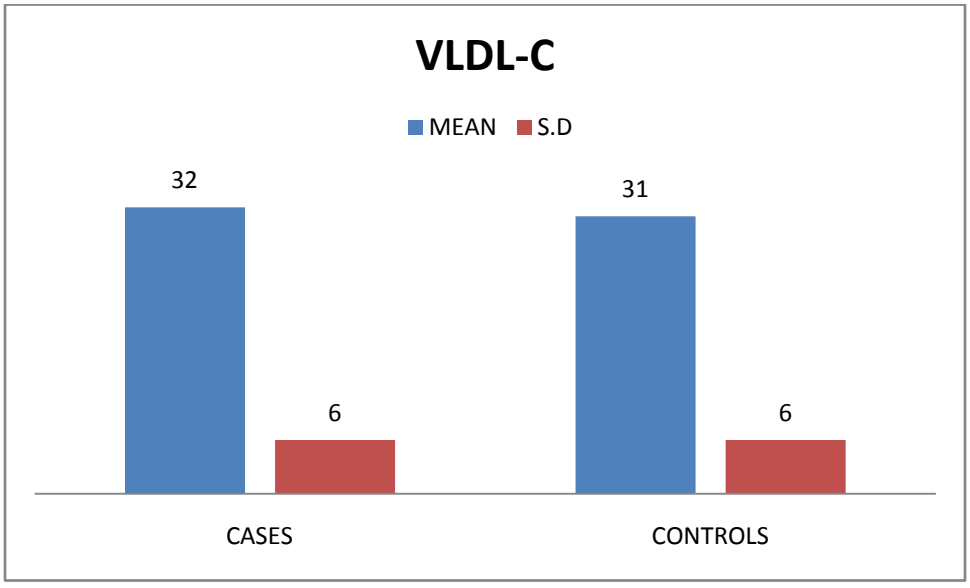

Fig. 19

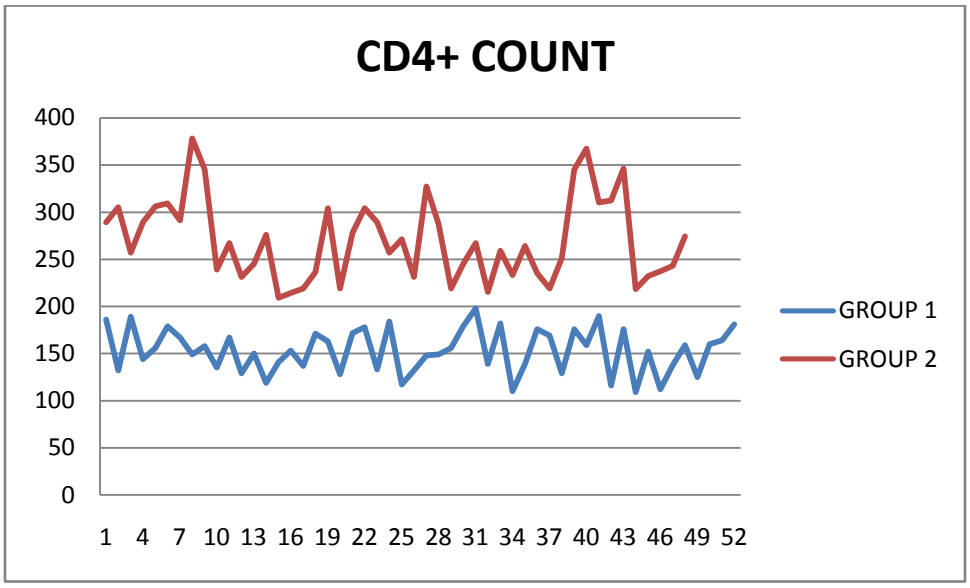

Fig. 20

Group 1- cases with dyslipidemia. Group 2- cases without dyslipidemia

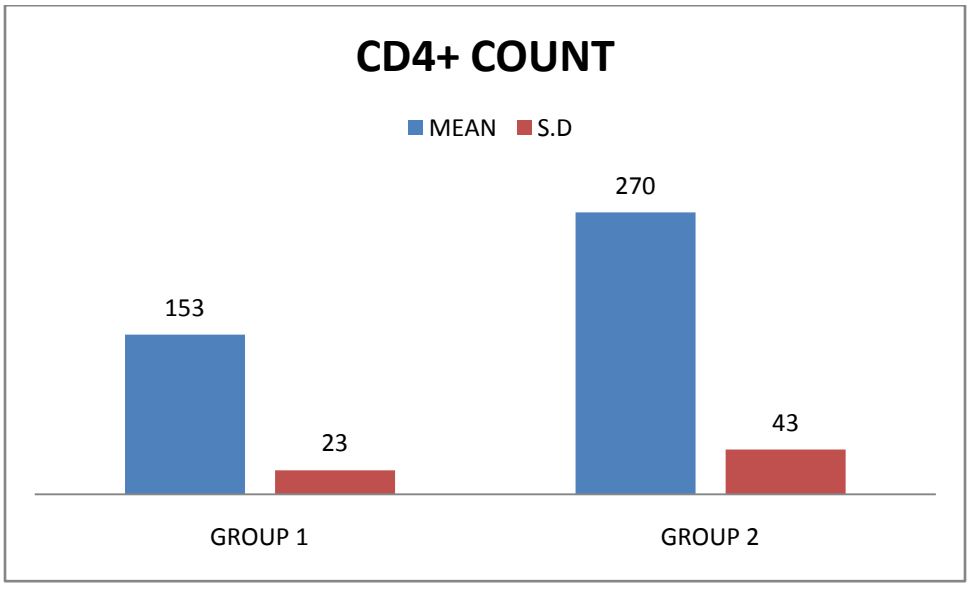

Fig. 21

LIPID PROFILE OF CASES AND CONTROLS:

Parameter(mg/dl)	Cases	Controls	P value
Triglycerides	174±75	133±35	<0.001
Total cholesterol	167±11	183±8	<0.001
LDL-C	114±28	84±9	<0.001
HDL-C	47±6	49±3	0.0027
VLDL-C	32±6	31±6	0.22

Note: All values are in mean ± standard deviation

Table 9: Lipid Profile of Cases and Controls

HIV patients with dyslipidemia has significantly lower CD4+ count (mean 153±23) when compared to patients with normal lipid profile (mean 270±43).

DISCUSSION

In this study, including 100 HIV positive cases and 100 HIV negative controls we observed a significant increase in triglycerides, LDL-C and significant decrease in total cholesterol and HDL-C levels.

HIV infection causes a specific pattern of derangement in lipid profile, resulting from a combination of increased production and decreased clearance of lipoproteins.

It has been found that out of 18 differentially expressed proteins in HIV infected cells, six kinases/enzymes are expressed exclusively in HIV infected cells. They are (CO3, P3C2B, FAS, GPX1, ACSL1, and KPCB), also there is slight downregulation of one isomerase PDIA3 after chronic HIV infection.

HIV infection alone without any influence of human genetic factors or of antiretroviral drugs induces these novel cellular proteins and enzymes. These further increase the quantity of LDL, secrete TGs, enhance fatty acid synthesis, alter the lipid transport, metabolism and oxidize lipids.

The main target of HIV infection is CD4+ T lymphocyte because of affinity of the virus toward the CD4+ surface marker. There is a gradual decline in CD4+ T lymphocyte levels resulting in increased risk for development of opportunistic infections.

Secondary acute infectious disease has been more associated with lipid abnormalities, which causes dyslipidemia independent of infectious agent and are brought about by various cytokines regulating the immune response to infection.

Hypertriglyceridemia was the first reported dyslipidemia in HIV infected patients. Hypo HDL- cholesterolemia, Hyper LDL- cholesterolemia and hypocholesterolmia has also been reported.

The hypertriglyceridemia correlates with opportunistic infections and to interferon-α. Interferon-α increases triglycerides by two mechanisms

1. Decrease in TGL clearance.
2. Increase in de novo hepatic lipogenesis and VLDL production.

This hepatic lipogenesis is stimulated by three cytokines.

1. Tissue necrotic factor- alpha(TNF-α)
2. Interleukin 1 and 6(IL-1 & IL-6)
3. Interferon-alpha(IFN-α)

Acute infections may increase TGL by the way of steroids or cytokines other than interferon-α or TNF-α. TNF-α is also found to play a role in per oxidation of plasma lipoproteins and lipids by stimulating the production of reactive oxygen species. Lipid per oxidation explains the alterations in cholesterol metabolism and these changes have significant effect in immune dysfunction in HIV patients.

CONCLUSION

- From the above findings, it is apparent that HIV infection induces changes in lipid profile, which should be measured to determine the high risk patients of myocardial infection before enrollment of HAART.
- All HIV infected persons should have their fasting lipid profile done before starting HAART with periodic repetitions thereafter since significant increases in plasma triglycerides and total cholesterol levels, are often associated with lipodystrophy and impaired glucose tolerance are seen in HIV patients on HAART.
- Therefore lipid profile serves as a good index for disease progression, intervention and management of HIV patients.

ABBREVIATIONS

HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency virus
WHO	World health organization
HAART	Highly active antiretroviral therapy
ART	Anti retroviral therapy
TGL	Triglyceride
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein
TC	Total cholesterol
Lp-a	Lipoprotein a
NCEP ATP	National cholesterol education programme adult treatment panel

PROFORMA

Name: Age / Sex: Occupation:

Presenting complaints:

Past History:

H/o DM, HT, drug intake, CAD, Thyroid disorders

Clinical Examination**:**

General Examination:

Consciousness, Pallor, jaundice, Clubbing, Lymphadenopathy,

Vitals:

PR, BP, RR, SpO2.

Systemic examination:

CVS: RS: ABDOMEN: CNS:

Laboratory investigations:

Lipid profile, FBS, PPBS, TSH

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MASTER CHART - CASES

S.NO	AGE	SEX	TGL	T.C	LDL	HDL	VLDL	CD4
1	34	M	132	169	89	49	20	289
2	31	M	124	178	101	43	31	305
3	28	M	120	161	127	54	37	186
4	39	F	267	157	121	49	28	257
5	27	M	273	186	129	46	37	289
6	35	M	126	169	128	51	40	306
7	33	M	117	167	110	45	34	309
8	36	M	294	164	165	54	23	132
9	38	M	132	178	105	38	33	189
10	21	F	253	168	117	48	41	291
11	32	F	275	156	119	44	34	144
12	30	M	240	148	110	49	28	378
13	24	M	110	169	179	53	20	156
14	33	M	98	149	89	48	27	345
15	35	M	283	147	97	55	31	179
16	21	M	116	170	103	47	37	239
17	28	F	129	178	110	32	29	167
18	29	M	139	143	121	50	36	149
19	31	M	98	162	85	53	34	267
20	34	F	128	171	168	48	32	158
21	38	M	292	156	173	51	38	135
22	32	M	131	177	94	43	39	231
23	37	F	127	178	119	33	29	167
24	28	M	179	173	121	49	37	129
25	31	M	343	164	87	53	31	245
26	36	F	122	179	76	47	36	276
27	29	M	281	172	105	49	37	150
28	37	F	129	166	114	54	42	209
29	36	F	119	162	120	52	32	214
30	31	M	277	160	83	50	39	119
31	34	M	134	171	161	54	30	141
32	29	M	259	169	114	34	35	153
33	22	M	121	163	109	47	37	219
34	39	M	110	174	98	49	32	137
35	40	M	99	172	79	50	27	171

36	23	F	114	161	106	51	23	236
37	35	F	285	170	110	49	27	163
38	39	M	135	177	124	54	29	304
39	32	F	280	165	166	53	30	128
40	27	F	125	167	120	39	32	172
41	35	M	130	174	119	51	19	219
42	39	M	131	175	113	47	36	278
43	26	M	127	179	169	43	34	178
44	21	M	239	178	71	46	36	304
45	29	M	295	171	84	44	38	133
46	38	M	234	170	93	48	34	289
47	25	F	129	174	90	31	29	184
48	39	M	110	178	91	49	26	257
49	22	M	103	174	99	53	20	117
50	32	M	117	179	84	50	14	271
51	38	M	131	179	118	49	34	231
52	31	M	114	170	79	48	33	327
53	35	M	278	176	127	54	37	132
54	34	M	131	178	106	55	39	287
55	38	F	126	179	89	46	36	219
56	32	F	284	175	94	43	35	148
57	36	M	131	179	110	49	39	245
58	31	M	110	174	108	35	20	149
59	37	M	119	179	112	49	29	267
60	35	M	121	176	172	47	26	156
61	29	M	132	170	129	45	31	215
62	34	M	290	179	88	54	32	179
63	39	M	123	179	79	53	26	259
64	37	F	131	170	80	33	27	198
65	32	M	125	176	84	55	30	233
66	39	M	119	141	123	47	32	139
67	36	M	127	178	113	44	31	264
68	35	M	298	147	119	43	35	182
69	39	M	135	179	123	42	37	235
70	28	M	131	148	186	48	39	110
71	35	F	276	140	100	49	37	139
72	31	M	244	149	98	36	32	176
73	38	M	139	148	91	43	39	219
74	34	M	282	147	87	54	30	169

75	39	M	128	174	84	55	38	251
76	34	F	240	170	99	51	29	345
77	39	M	372	179	115	43	35	129
78	35	M	112	159	126	54	25	367
79	31	M	130	148	118	52	37	310
80	39	F	89	179	179	49	30	176
81	34	F	291	171	120	47	33	159
82	36	M	117	151	123	34	36	190
83	32	M	131	178	107	48	23	312
84	23	M	139	172	103	47	31	116
85	29	M	243	175	188	47	36	176
86	34	M	127	179	101	49	30	346
87	38	F	286	164	174	44	32	109
88	34	M	134	168	119	54	36	218
89	39	M	125	161	116	40	30	152
90	36	F	129	146	92	50	31	232
91	27	M	279	149	90	51	47	112
92	33	F	98	146	87	53	29	237
93	37	M	297	170	108	52	35	138
94	39	M	103	165	119	48	36	243
95	38	M	112	160	116	46	33	159
96	35	M	135	149	129	49	32	274
97	32	F	127	178	120	33	38	125
98	31	M	134	174	191	50	30	160
99	30	M	258	171	110	53	34	164
100	34	M	222	169	99	37	39	181

MASTER CHART - CONTROLS

S.NO	AGE	SEX	TGL	T.C	LDL	HDL	VLDL
1	35	M	134	165	76	46	40
2	30	M	132	172	73	49	34
3	30	M	138	170	72	52	23
4	39	M	129	164	80	51	33
5	26	M	134	183	85	49	41
6	36	M	231	190	87	46	34
7	32	M	139	184	89	54	28
8	37	M	129	180	85	48	20
9	39	M	132	163	76	52	27
10	20	F	138	167	78	47	31
11	33	F	132	177	95	49	37
12	29	M	134	174	95	46	29
13	25	M	132	161	86	50	36
14	34	M	117	180	76	52	34
15	36	M	134	185	73	48	32
16	20	M	140	162	99	46	32
17	27	F	128	171	97	51	27
18	30	M	92	174	87	49	23
19	32	M	104	169	66	46	27
20	35	F	249	170	67	55	29
21	37	M	116	170	69	52	30
22	33	M	86	168	70	46	32
23	36	M	95	167	72	49	19
24	29	M	108	186	77	51	36
25	30	M	112	180	78	49	34
26	37	F	234	174	83	46	36
27	28	M	96	172	87	54	38
28	36	F	88	188	89	52	34
29	37	F	95	188	94	42	29
30	30	M	101	172	97	46	26
31	35	M	86	174	84	45	20
32	28	M	98	172	87	53	20
33	23	M	92	180	78	46	29
34	40	M	246	163	95	52	26
35	39	M	94	161	94	50	31

36	24	F	124	174	97	55	32
37	36	F	133	175	99	46	26
38	38	M	130	167	73	49	27
39	33	F	141	190	76	51	30
40	26	F	148	162	84	47	32
41	34	M	121	168	94	54	31
42	40	M	129	174	97	46	35
43	27	M	126	167	85	48	37
44	23	M	144	188	80	53	39
45	28	M	129	190	72	54	37
46	35	M	124	184	76	45	32
47	27	M	139	163	89	49	39
48	38	M	239	175	87	53	30
49	23	F	106	162	76	46	25
50	34	M	112	185	78	48	37
51	37	M	109	163	72	51	30
52	30	M	117	177	71	47	33
53	36	M	148	160	91	49	36
54	33	M	138	168	93	52	23
55	39	F	124	179	95	47	31
56	31	F	131	165	94	54	36
57	37	M	103	189	84	46	30
58	30	M	139	166	78	49	32
59	38	M	140	182	97	51	36
60	36	M	142	168	99	54	30
61	28	M	135	172	92	47	31
62	35	M	123	184	81	49	47
63	40	M	241	162	83	51	29
64	38	M	137	166	77	53	35
65	31	M	138	176	75	46	36
66	38	M	134	174	86	48	33
67	37	F	135	188	89	50	32
68	34	M	134	164	95	54	38
69	40	M	132	178	97	49	30
70	27	M	131	182	99	53	34
71	36	F	230	180	76	55	26
72	30	M	146	162	71	45	20
73	39	M	133	164	73	49	14
74	33	M	138	168	75	46	34

75	38	M	131	170	77	51	33
76	35	M	136	180	81	49	37
77	37	M	125	172	89	53	39
78	34	M	139	182	91	48	36
79	32	M	139	169	76	51	35
80	38	F	148	174	79	54	39
81	35	F	141	183	77	47	20
82	37	M	104	176	99	53	29
83	32	M	137	167	97	49	26
84	24	M	139	184	93	51	31
85	30	M	138	163	91	50	32
86	34	M	134	175	82	45	26
87	38	M	132	169	86	54	27
88	35	M	139	178	75	46	30
89	39	M	233	183	82	51	32
90	36	F	118	177	91	50	31
91	28	M	122	160	93	46	35
92	33	F	101	165	97	49	37
93	39	M	132	176	77	51	39
94	39	M	104	186	81	43	37
95	38	M	92	167	92	50	32
96	35	M	109	183	99	47	23
97	32	M	103	164	82	49	37
98	31	M	103	170	88	53	29
99	30	M	136	162	76	46	39
100	34	M	102	167	80	44	21