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Assessment of Lipid profile in relation to antiretroviral therapy in HIV Population in Western Maharashtra

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Abstract
There were several studies done for the prevalence and pattern of dyslipidaemia in HIV patient but most of them were conducted in urban setting and there was no study done from western HIV population in India in rural set up to show the lipid profile in HIV patient.
Keeping in view of this, the biochemical abnormalities associated with lipid metabolism, our research was inclined to assess the lipid profile in HIV patients. This study will also add our knowledge that whether the lipid profile has any role in the disease progression of HIV AIDS.
Keywords: *Lipid profile ART, HIV, dyslipidemia, biochemical abnormalities.*

Introduction

Patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) frequently present alterations in lipid metabolism due to infection with HIV itself, including elevated serum concentrations of triglycerides and low levels of total cholesterol.[1] The introduction of antiretroviral therapy (ART) in the mid-1990s led to substantial improvement in the prognosis of HIV/AIDS patients, with a reduction in morbidity and mortality due to opportunistic diseases and consequent improvement of the patient's quality of life.[2–7]

Lipid alterations in patients with HIV/AIDS caused by the infection itself had been reported before the implementation of ART.[1,8] In this respect, serum triglyceride concentrations were higher and the levels of total cholesterol, LDL-c and HDL-c were lower in HIV-seropositive patients receiving no ART when compared to uninfected controls. These alterations were detected in patients infected with different HIV-1 subtypes. Low serum concentrations of HDL-c can be used as a marker of chronic inflammatory activity[9].

Recent evidence suggests that HIV infection, even with treatment, increases the risk of coronary

heart disease (CHD) and that both chronic inflammation and traditional risk factors play key roles in HIV associated CHD.[10]

Hypertriglyceridemia is a common finding in AIDS and is independent of the degree of wasting.[11]

Infection can increase serum triglyceride (TG) level by decreasing the clearance of circulating lipoprotein, which thought to be due to reduced lipoprotein lipase or by stimulating lipid synthesis in liver through increase in either hepatic fatty acid synthesis or re-esterification of fatty acid derived from lipolysis.[12]

Protease inhibitors of ART drug regimens are associated with elevated levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglycerides, as well as insulin resistance and abdominal fat accumulation, factors that promote inflammation and hence atherosclerosis. Other groups of ART drug regimens have similar atherogenic effects, albeit to varying degrees Long-term ART exposure has been associated with increased risk of dyslipidemia and CHD, particularly for combinations that are protease inhibitor-based.

In addition, ART may indirectly play a role in inflammation through its effects on metabolism though it is not clear, if antiretroviral drugs directly cause inflammation[13]

Initial concerns of the increased rates of myocardial infarction arising as a result of dyslipidemia in HIV infected patients on antiretroviral (ARV) have been confirmed by studies such as the D.A.D study, a large, mutlicohort study that showed association between exposure to antiretroviral therapy and increased risk of myocardial infarction.

Endothelial function is also found to be abnormal in HIV infected patients. Changes in platelet reactivity among HIV infected patients also reported and increased rates of insulin resistance and diabetes is a well described side effect of exposure to some of the ARV. All of these factors act in combination with dyslipidemia to increase overall cardiovascular risk of HIV infected patients on ARV[14].

There were several studies done for the prevalence and pattern of dyslipidaemia in HIV patient but most of them were conducted in urban setting and there was no study done from western HIV population in India in rural set up to show the lipid profile in HIV patient.[12]

Keeping in view of this, the biochemical abnormalities associated with lipid metabolism, our research was inclined to assess the lipid profile in HIV patients. This study will also add our knowledge that whether the lipid profile has any role in the disease progression of HIV AIDS.[15]

Aim

- To assess the lipid profile in HIV PTS.

Objectives

1. To estimate lipid profile in HIV pt.
2. To compare the lipid profile in ART treated and not treated HIV pts.

Material and Methods

- **Study Setting:** University Medical college, Tertiary Care Hospital.
- **Study design**: Comparative prospective study
- **Study duration**: From January 2010 to July 2011
- **Ethical committee**
- Research protocol was submitted to institutional ethics committee for scientific and ethical approval.
- **Detailed Research Plan:**
- **Study population**

HIV positive, adult (age group 15-49) male and female patients attending ART OPD (Out Patient Department) and In Patient Department (IPD) were the study participants.

Patients which were included in this study were diagnosed HIV positive as per NACO guidelines using three spot tests COOMBS AIDS, TRILINE, QUALPRO.

Proper history was taken and clinical examination of 5000 participants was done. Out of these patients, 1000 cases who had history of cardiovascular disease and clinical manifestations of cardiovascular disease were included in present study with written consent.

A detailed clinical profile including detailed history, general physical examination and systemic examination was done for each patient with special emphasis on cardiovascular system.

Line of investigation obtained for all the participants:

- ECG, CHEST X-RAY, lipid profile and 2D-ECHO was done in all patients and
- CT BRAIN ,COLOUR DOPPLER was done in relevant cases only.
- Routine investigations like HB/CBC/RFT was used only for primary evaluation.

All patients were evaluated for their CD4 count and were analyzed for different cardiac abnormalities.

Collection of Blood Sample

About 2 ml of fasting blood sample was collected by venous puncture with all aseptic precautions in a plain vacutainer. It was allowed to clot for one hour for separation of serum. The serum and plasma was separated by centrifugation at 2500 rpm for 5 minutes at room temperature. Serum and plasma were ensured to be free from hemolysis and turbidity. Separated serum sample was subjected towards estimation of following parameters

Lipid Profile	Method
i) Serum Total Cholesterol (CHO)	Cholesterol Oxidase- peroxidase (CHOD-PAP) method
ii) Serum Triglycerides (TG)	Glycerol phosphate oxidase method
iii) Serum High Density Lipoprotein Cholesterol(HDL) –direct	Enzymatic method
iv)Serum Low Density Lipoprotein Cholesterol (LDL)	Calculated by Friedewald's equation
v) Serum Very Low Density Lipoprotein Cholesterol (VLDL)	Calculated by Friedewald's equation

We used this information for study purpose only and not disclosed elsewhere.

Inclusion criteria

HIV positive adult (age group 15-49) male and female patients, newly diagnosed and previously known cases of HIV considered who are diagnosed at VCTC center under ART department in our hospital.

Exclusion criteria

In the present study by history and clinical finding following cases were excluded

1) Congenital heart diseases

2) Known case of valvular heart disease

3) Known case of ischemic heart disease before VCTC report positive.

Statistical Analysis

Statistical analysis was done with Scientific Package for Social Sciences (SPSS) version 20.0and MS Excel 2007 spreadsheet.

Student's t test and $\chi 2$ test, Pearson's correlation coefficient, were used for analysis.

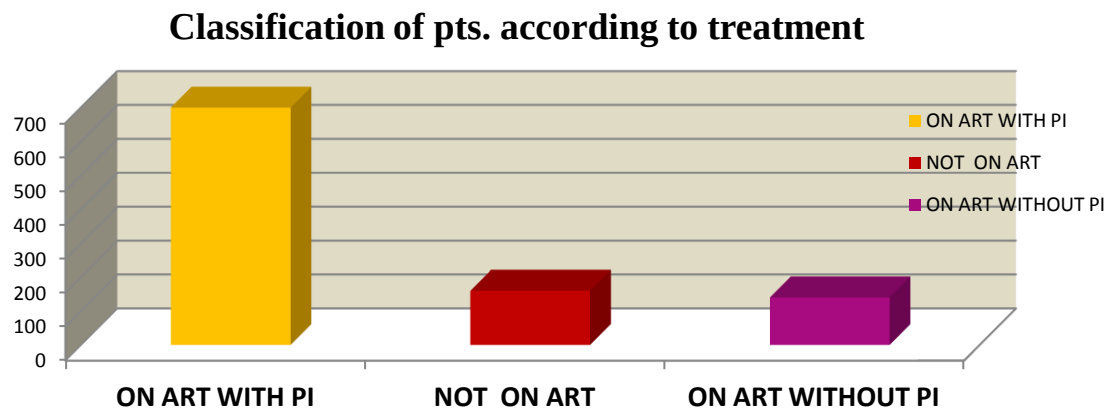
P value <0.05 will be considered statistically significant.

Observations and Result

1. Classification of pts. according to antiretroviral treatment status

Table No 1		
Drug use	**No.of pts.**	**%**
On ART with PI	700	70%
On ART without PI	140	14%
Not on ART	160	16%

In present study, 700 pts. were on HAART containing PI out of total 1000 patients, and 140 pts.on HAART not containing PI. Number of patients who were not on HAART were 160.



2. Relation between antiretroviral therapy and lipid profile

Table no.2 (Discussion) Relation between antiretroviral therapy and lipid profile in present study and paulo sérgio ramos dearaújo et.al comparison

Table No 2						
	TOTAL CHOLESTEROL		HIGH DENSITY CHOLESTEROL		TRIGLYSERIDE	
	> or = 220 mg/dl		< 40 mg/dl,		> or = 150 mg/dl,	
	% pts. on ART(N=350) WITH PIs.	% pts.on in Paulo Sérgio Ramos dearaújo et.al [147]	% pts. on ART(N=350) WITH PIs.	% pts.on in Paulo Sérgio Ramos dearaújo et.al[147]	% pts. on ART(N=350) WITH PIs.	% pts.on in Paulo Sérgio Ramos dearaújo et.al [147]
Pts. on ART (n=350) with PIs.	75.71	27.2	40	58.1	47.14	64.5
Pts.on ART (N=70) without PIs.	42.85	17.3	14.28	53.6	35.71	43.6
Pts.not on ART (N=80)	31.25	14.2	12.5	59.3	31.5	30.1
Total n=500	64	-----	32	----	43	-----

Total Cholesterol

- In present study, 75.71% pts. On HAART with PI drugs has raised total cholesterol > or = 220mg/dl.
  Paulo Sérgio Ramos dearaújo et.al [147] in 2000 study, 27.2% pts. has raised total cholesterol >or=220mg/dl.
  In Behrens, Georga et.al[148] study 71% pt has total cholesterol >or=220mg/dl .in pts. on HAART with PI drugs.
- In group of pts. on HAART without PIs, in present study, 42.85% pts. has total cholesterol >or=220mg/dl.
  In Paulo Sérgio Ramos dearaújo et.al study [147] has total cholesterol >or=220mg/dl.in 17.3 % pts.
  In Salami AK,et.al[133] study, 31% has total cholesterol >or=220mg/dl in pts. on HAART without PIs.
- In group of pts. not on HAART, 31.25% pts. has total cholesterol >or=220mg/dl.
  Paulo Sérgio Ramos dearaújo et.al study [147] has total cholesterol >or=220mg/dl in 14.2% pts, in group of pts.not on HAART.
  In Mark Mascolini[146] study, 24.6% pts. has total cholesterol >or=220mg/dl in group of pts.not on HAART .

In present study, no.of pts.having total cholesterol >or=220mg/dl is seen in > 70% of pts.

In present study, total cholesterol is >or=220mg/dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. **(**Chi-Sq =143.338 P-Value =0.001).

High Density Cholesterol

- In present study, 40% pts. on HAART with PI drugs has high density cholesterol (HDL) <40mg /dl.
  In Paulo Sérgio Ramos dearaújo et.al[147] in 2000 study[147], 58.15% pts. has high density cholesterol(HDL) < 40mg /dl.
  In Bente M Bergersen et.al[150] study, 35.5% pts. has high density cholesterol (HDL)<40mg /dl.
- In group of pts.on HAART without PI, in present study, 14.28% pts. has high density cholesterol(HDL) <40mg /dl.
  In Paulo Sérgio Ramos dearaújo et.al study [147], 53.6 % pts. in group of on HAART without PIs has high density cholesterol(HDL) < 40mg /dl.
  In Bente M Bergersenet.al [150] study, 7.5% pts. in group on HAART without PIs has high density cholesterol (HDL) <40mg /dl.
- In group of pt. not on HAART, 12.5% pts. has high density cholesterol(HDL)<40mg /dl.
  In Paulo Sérgio Ramos dearaújo et.al study [147], 59.3% pts. in group not on HAART has high density cholesterol(HDL) < 40mg/dl.
  In Bente M Bergersen et.al[150] study, 7.5% pts. has high density cholesterol (HDL) <40mg /dl. in group of pts.not on HAART.
  In present study, compared to other studies, less no.of pts. having high density cholesterol (HDL) <40mg /dl.
  In present study, high density cholesterol (HDL) < 40mg /dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. (Chi-Sq= 22.619 P-Value = 0.001).

Triglyceride

- In present study, 47.14 % pts.on HAART with PIs drugs has triglyceride (TG) > or = 150 mg/dl.
  In Paulo Sérgio Ramos dearaújo et.al [147] in 2000 study [147], 64.5% pts. on HAART with PIs drugs has triglyceride (TG) > or = 150 mg/dl.
  In Salami AK, et.al [9] study, 79% pts. on HAART with PIs drugs has triglyceride (TG) > or = 150 mg/dl.
- In group of pts. on HAART without PIs, in present study, 35.71% pts. has triglyceride (TG) > or = 150 mg/dl.
  In Paulo Sérgio Ramos dearaújo et.al study[147], 43.6 % pts. on HAART without PIs has triglyceride (TG) > or = 150 mg/dl.
  In Salami AK, et.al [133] study, 54% pts. on HAART without PI has triglyceride (TG) > or = 150 mg/dl.
- In group of pts.not on HAART, 31.5% pts.has triglyceride (TG) > or = 150 mg/dl.
  In Paulo Sérgio Ramos dearaújo et.al study[147] pts.not on HAART, 30.1% has triglyceride (TG) > or = 150 mg/dl .
  In Salami AK, et.al [133] study pts.not on HAART, 17% has triglyceride (TG) > or = 150 mg/dl.
  In present study, compared to other studies, less no.of pts. having triglyceride (TG) > or = 150 mg/dl.
  In present study, triglyceride (TG) > or = 150 mg/dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. (Chi-Sq = 16.946 ,P-Value = 0.001).
  Paulo Sérgio Ramos dearaújo et.al in 2000 study [147] did not included low density cholesterol.

Table No.3 (Observation and Results)

- **Relation between antiretroviral therapy and lipid profile---**

Table 3

	TOTAL CHOLESTEROL				LOW DENSITY CHLESTEROL				HIGH DENSITY CHOLESTEROL				TRIGLYSERIDE			
	> or = 220 mg/dl		<220 mg/dl		> or = 140 mg/dl		<140 mg/dl		< 40 mg/dl,		>40 mg/dl,		> or = 150 mg/dl,		<150 mg/d l,	
	No pt	%	No PT	%	No Pt	%	No Pt	%	No Pt	%	No Pt	%	No Pt	%	No Pt	%
ON ART (N=700) WITH PI	530	75.71	170	24.28	300	42.85	400	57.14	280	40	420	60	330	47.14	370	25.85
ON ART (N=140) WITHOUT PI	60	42.85	80	57.14	70	50	70	50	20	14.28	120	85.71	50	35.71	90	64.28
NOT ON ART (N=160)	50	31.25	110	68.75	40	25	120	75	20	12.5	140	87.5	50	31.5	110	68.75
Total(n=1000)	640	64	360	36	410	41	590	59	320	32	680	68	430	43	570	57

1. **In pt.on HAART with PI**, total cholesterol > or = 220 mg/dl was in >75%.

2. In pts.on HAART without PI and pts. not on HAART, total cholesterol > or = 220 mg/dl which was seen in <50% pts. (42.85% and 31.25% respectively).

3. In total HIV positive pts,
64%pts. had total cholesterol > or = 220 mg/dl ,
41% had LDL > or = 140 mg/dl,
32 % pts. had HDL < 40 mg/dl,
43% pts. had triglyceride > or = 150 mg/dl.

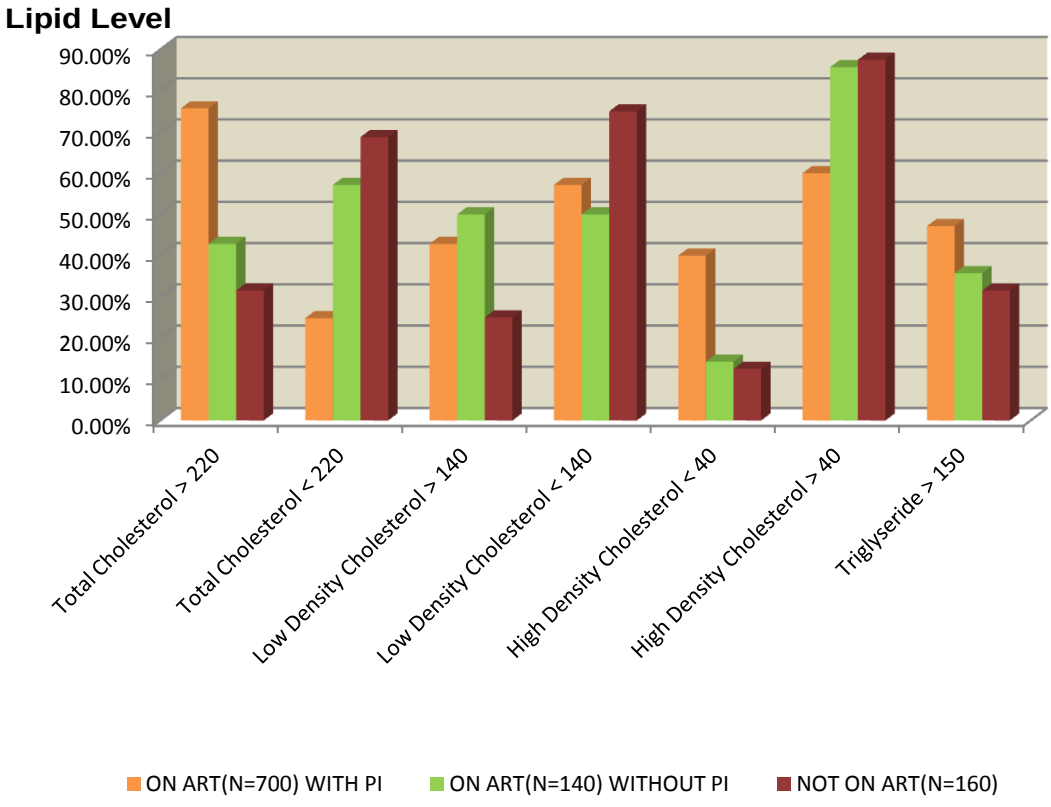


Table No.4 (Discussion): Relation between antiretroviral therapy and low density cholesterol in present study and other studies comparison

TABLE NO.4						
	Pts.on ART(N=700) with PIs.		**Pts.on ART (N=140) without PIs.**		**Pts. not on ART(N=160)**	
	present study % of pts.	**Behrens, Georga et.al % of pts.[148]**	**present study % of pts.**	**Mark Mascolini[146] % of pts.**	**present study % of pts.**	**Mark Mascolini[146] % of pts.**
Low density chlesterol > or = 140 mg/dl	42.85	54	50	46.4	25	21

- In present study, low density cholesterol > or = 140 mg/dl is seen in 42.85% pts. on ART.
  With PIs Behrens, Georga et.al [148] Study, 54% has low density cholesterol> or = 140 mg/dl.
- In on ART without PIs group, 50% pts. has low density cholesterol > or = 140 mg/dl.
  Mark Mascolini study [146] In on ART without PIs group pts, 46.4% has low density cholesterol > or = 140 mg/dl.
- In pts.group not on ART, in present study, 25% pts. has low density cholesterol > or = 140 mg/dl.
  Mark Mascolini study[146] In pts.group not on ART, 21% pts. has low density cholesterol> or = 140 mg/dl.
  Present study results and Mark Mascolini study results in pts. group not on ART and on ART without PI group approximately similar .
  In present study no.of pts. having low density cholesterol > or = 140 mg/dl is less.
  In present study, cholesterol > or = 140 mg/dl in all three groups On HAART with PIs drugs, on HAART without PIs, not on HAART (Chi-Sq = 68.737,P-Value = 0.001).
  In present study, maximum no.of pts. has increase total cholesterol. Lipid abnormalites maximum in pts. On HAART with PIs as compared to other two group not on ART and on ART without PIs.

Discussion

In present study, 700 pts. were on HAART containing PI out of total 1000 patients, and 140 pts. on HAART not containing PI. Number of patients who were not on HAART were 160.

Total Cholesterol

- In present study, 75.71% pts. On HAART with PI drugs has raised total cholesterol > or =220mg/dl. Paulo Sérgio Ramos dearaújo et.al in 2000 study, 27.2% pts. has raised total cholesterol >or=220mg/dl.
- In group of pts. on HAART without PIs, in present study, 42.85% pts. has total cholesterol >or=220mg/dl. In Paulo Sérgio Ramos dearaújo et.al study has total cholesterol >or=220mg/dl. in 17.3 % pts.
- In group of pts. not on HAART, 31.25% pts. has total cholesterol >or=220mg/dl.
  Paulo Sérgio Ramos dearaújo et.al study has total cholesterol >or=220mg/dl in 14.2% pts, in group of pts. not on HAART.
  In present study, no.of pts. having total cholesterol >or=220mg/dl is seen in > 70% of pts.
  In present study, total cholesterol is >or=220mg/dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. **(**Chi-Sq =143.338 P-Value =0.001).

High Density Cholesterol

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  In Paulo Sérgio Ramos dearaújo et.al study, 53.6 % pts. in group of on HAART without PIs has high density cholesterol(HDL) < 40mg /dl.
- In group of pt. not on HAART, 12.5% pts. has high density cholesterol(HDL)<40mg /dl.
  In Paulo Sérgio Ramos dearaújo et.al study, 59.3% pts. in group not on HAART has high density cholesterol(HDL) < 40mg/dl.

In present study, compared to other studies, less no.of pts. having high density cholesterol (HDL) <40mg /dl.

In present study, high density cholesterol (HDL) < 40mg /dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. (Chi-Sq= 22.619 P-Value = 0.001).

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- In group of pts.not on HAART, 31.5% pts.has triglyceride (TG) > or = 150 mg/dl.
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In present study, triglyceride (TG) > or = 150 mg/dl in all three groups on HAART with PIs drugs, on HAART without PIs, not on HAART. (Chi-Sq = 16.946, P-Value = 0.001).

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2.In pts.on HAART without PI and pts. not on HAART, total cholesterol > or = 220 mg/dl which was seen in <50% pts. (42.85% and 31.25% respectively).

3. In total HIV positive pts,

64% pts. had total cholesterol > or = 220 mg/dl ,
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32 % pts. had HDL < 40 mg/dl,
43% pts. had triglyceride > or = 150 mg/dl.

- In present study, low density cholesterol > or = 140 mg/dl is seen in 42.85% pts. on ART. With PIs Behrens, Georga et.al Study, 54% has low density cholesterol> or = 140 mg/dl.
- In on ART without PIs group, 50% pts. has low density cholesterol > or = 140 mg/dl.
  Mark Mascolini study In on ART without PIs group pts, 46.4% has low density cholesterol > or = 140 mg/dl.
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  Present study results and Mark Mascolini study results in pts. group not on ART and on ART without PI group approximately similar.
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In present study, cholesterol > or = 140 mg/dl in all three groups On HAART with PIs drugs, on HAART without PIs, not on HAART (Chi-Sq = 68.737, P-Value = 0.001).

In present study, maximum no.of pts. has increase total cholesterol. Lipid abnormalites maximum in pts. On HAART with PIs as compared to other two group not on ART and on ART without PIs.

Discussion

1.**In pt.on HAART with PI** ,total cholesterol > or = 220 mg/dl was in >75%.

2.In pts.on HAART without PI and pts. not on HAART, total cholesterol > or = 220 mg/dl which was seen in <50% pts. (42.85% and 31.25% respectively).

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32 % pts. had HDL < 40 mg/dl,

43% pts. had triglyceride > or = 150 mg/dl.

In present study lipid levels were altered in HIV infected patients and there were varied relationship between different lipid levels in WHO clinical stage.

Endothelial function is also found to be abnormal in HIV infected patients. Changes in platelet reactivity among HIV infected patients also reported and increased rates of insulin resistance and diabetes is a well described side effect of exposure to some of the ARV. All of these factors act in combination with dyslipidemia to increase overall cardiovascular risk of HIV infected patients on ARV.

Hypertriglyceridemia in patients on antiretroviral therapy is common among patients taking Protease inhibitors as they increase the hepatic triglyceride synthesis by increased expression of key enzymes involved in its synthesis. There is impaired uptake of triglycerides in the adipocytes which leads to increases in their levels.

Antiretroviral drugs are responsible for reduced expression of LDL receptors, thus reducing fat storage and increasing free fatty acid plasma levels The presence of an atherogenic lipid profile in HIV patients on antiretroviral therapy makes these patients more susceptible to cardiovascular events. A longer duration of antiretroviral therapy is associated with greater chances of coronary artery stenosis due to dyslipidemia and due to the metabolic effects of HIV infection. This has lead to increased concerns of myocardial infarction in HIV patients. The presence of dyslipidemia in young patients makes them susceptible to subclinical coronary atherosclerosis is a major concern as lack of suspicion of coronary artery disease makes them prone to sudden grave consequences. The lack of an alternative to antiretroviral therapy makes it more difficult to protect and prevent these patients from dyslipidemia and thus future cardiac events. After initiation of antiretroviral therapy of fasting lipid profile is a must for HIV patients.

Conclusion

In present study, maximum no.of pts.has increase total cholesterol. Lipid abnormalites maximum in pts. On HAART with PIs as compared to other two group not on ART and on ART without PIs.

Implications

The presence of dyslipidemia in patients makes them susceptible to coronary atherosclerosis is a major concern as lack of suspicion of coronary artery disease makes them prone to sudden grave consequences. The lack of an alternative to antiretroviral therapy makes it more difficult to protect and prevent these patients from dyslipidemia and thus future cardiac events. Therefore we suggest, after initiation of antiretroviral therapy of fasting lipid profile is a must for HIV patients.

This study could predict the stage of HIV infection by measuring the changes in lipid profile.

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