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Biochemistry Section

Comparison of Serum Lipid Profile in HIV Positive Patients on ART with ART Naïve Patients

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ABSTRACT

Introduction: The widespread use of effective highly active antiretroviral therapy (HAART) in HIV patients has coincided with increasing reports of complications like HIV-associated lipodystrophy syndrome and the metabolic alterations, affecting the lipid and glucose metabolism. Evidences in support of lipodystrophy and dyslipidaemia associated with First- line HAART in our area is scarce. The aim of the present study was 1) to study and compare Lipid profile in HIV positive patients on ART with that of freshly diagnosed HIV positive patients who were yet to be started on ART. 2) To assess lipodystrophy syndrome in patients on ART.

Materials and Methods: Hundred newly diagnosed HIV positive patients who were yet to be started on ART were taken as controls (ART-Naïve).Hundred randomly selected HIV+ patients who were already on First-line ART regimen (Stavudine/Zudovudine + Lamivudine + Nevirapine) for more than 12 months were taken as cases (ART). This study was conducted for a period of 12 months at the VIMS ART centre, Bellary, Karnataka, India.

Results: There was a significant increase ($p<0.001$) in serum Total Cholesterol, LDL-C, TG, VLDL, Non-HDL -C & TC/HDL-C ratio in ART patients compared to ART-naïve patients. Of the 100 ART patients 23 had lipodystrophy syndrome (buffalo hump, abnormal fat deposition around neck & back, buccal fat resorption, increase in abdominal fat).

Conclusion: To conclude, it is evident from our study that there is increase in lipid profile (except HDL) in ART patients compared to ART Naïve group and 23 ART patients showed lipodystrophy syndrome. Hence it appears reasonable to measure fasting lipid levels before and 3-6 months after antiretroviral therapy is initiated or when ART regimen is changed.

Keywords: First line HAART regimen, Lipodystrophy syndrome, Lipid profile

INTRODUCTION

The introduction of an effective highly active antiretroviral therapy (HAART) in 1996 for the treatment of HIV infection has resulted in dramatic decline in the mortality and morbidity of people living with HIV [1]. However, the widespread use of effective ART has coincided with increasing reports of complications. The two main complications associated with ART are the HIV-associated lipodystrophy syndrome and the metabolic alterations affecting the lipid and glucose metabolism [2]. The lipodystrophy syndrome includes distressing morphologic changes in body habitus, characterized by redistribution of fat, with loss of fat from the extremities, buttocks & face (lipoatrophy), with or without fat accumulation in the abdomen due to an increase in visceral fat or more rarely an increase in the amount of fat in the neck (buffalo hump) or breasts. Besides these phenotypic changes a large proportion of patients develop insulin resistance and elevated plasma concentrations of LDL-Cholesterol, total cholesterol and triglycerides. The combination of hyperlipidemia, insulin resistance and visceral fat accumulation closely resembles the 'Metabolic Syndrome X' and has raised the concern that HIV-infected patients treated with ART may be at increased risk of developing premature coronary artery disease and diabetes [3-6].

The cause of metabolic disturbances and morphologic changes related to ART are not understood completely. The aetiology is likely to involve the effect of HIV per se as well as the direct and indirect effects of ART, superimposed on individual characteristics such as genetic predisposition, gender and age. This knowledge opens the door for individualized ART treatment, based on criteria in addition to HIV-1 viral and CD4-cell count [7].

HAART regimens typically include a combination of at least three drugs, such as different association of protease inhibitors (PI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTI) [8]. First-line HAART regimens as defined by World Health Organization (WHO) are largely used in resources- constrained countries and do not include Protease inhibitors (PI) [9]. Evidences in support of lipodystrophy and dyslipidaemia associated with First- line HAART in our area are scarce [10,11]. The aim of the present study was 1) to study and compare Lipid profile in HIV positive patients on ART with that of freshly diagnosed HIV positive patients who were yet to be started on ART. 2) To assess lipodystrophy syndrome in patients on ART.

MATERIALS AND METHODS

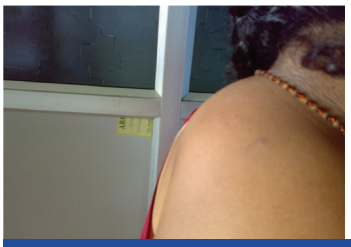
The participants were divided into two groups of 100 each. One group included individuals with newly diagnosed HIV-1 infection and was yet to be started on ART (ART-naïve group). The other group included HIV-positive individuals, who were already on ART for atleast 12 months (ART group). The first choice for first-line ART regimen was Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine (NVP) for patients with Hb > 8 g/dl. The second choice of First- line ART regimen was Stavudine (d4T) + Lamivudine (3TC) + Nevirapine (NVP) for patients with Hb < 8 g/dl. NVP was substituted with Effavirenz (EFV) for patients with Tuberculosis or toxicity to NVP.

This study was conducted for a period of 12 months at the VIMS ART centre, Bellary, Karnataka, India. An informed consent was obtained from all the participants and they were assured of confidentiality of their identity. The study was approved by the Institutional Ethical Committee.

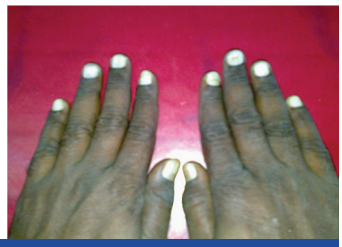
All the participants were adults of more than 20 yrs of age and included both males and females. The treatment adherence rate for the group on ART was > 95%. Level of adherence was assessed by verbal administration of a standard series of questions adapted from Adult AIDS Clinical trials group (AACTG) adherence instruments. The 95% rate of adherence is referable to 4-day recall data [12]. None of the participants were on lipid –lowering drugs at the time of their enrollment.

Sl. No	Characteristics	ART-Naïve (n=100)	ART (n=100)	p-value
1.	Age (years) (M± SD)	35.75 ± 9.05	36 ± 8.99	0.8448
2.	Men (%)	67	60	NA
3.	Weight (Kgs)	42.77 ± 8.61	48.9 ± 11.11	0.0001
4.	Mean duration on ART (months)	NA	25.3 ± 13.17	NA
5.	Tuberculosis	29	14	NA
6.	VDRL	05	00	NA
7.	Hbs Ag	09	00	NA

[Table/Fig-1]: Profile of ART-Naïve and ART patients



[Table/Fig-2]: Buffalo Hump



[Table/Fig-3]: White Nail Syndrome

Sl. No	Parameters	ART-Naïve (n=100)	ART (n=100)	p-value
1.	Total Cholesterol (mg/dl)	151.58 ± 29.41	191.95 ± 41.4	<0.0001
2.	HDL Cholesterol (mg/dl)	33.28 ± 7.78	34.59 ± 8.97	0.2713
3.	Total Chol/HDL ratio	4.65 ± 0.80	5.83 ± 1.61	< 0.0000
4.	LDL Cholesterol (mg/dl)	91.91 ± 23.32	127.36 ± 37.95	<0.0000
5.	Triglycerides (mg/dl)	133.78 ± 38.73	150.35 ± 42.78	0.0045
6.	VLDL (mg/dl)	26.60 ± 7.73	29.99 ± 8.21	0.0029
7.	Non-HDL cholesterol(mg/dl	118.29 ± 24.41	157.36 ± 38.77	<0.0000
8.	CD4 Count	123.95 ± 100.39	393.66 ± 160.24	<0.0001

[Table/Fig-4]: Lipid Profile & CD4 Count in ART-Naïve and ART patients

Sl. No	Parameters	ART-Naïve (n=100)	ART (n=100)	p-value
1.	Total Cholesterol (>200 mg/dl)	08	49	<0.0001
2.	HDL Cholesterol (<40 mg/dl)	81	68	0.0366
3.	Total Chol/HDL ratio (>5)	25	68	<0.0001
4.	LDL Cholesterol (>130 mg/dl)	05	48	<0.0001
5.	Triglycerides (>150 mg/dl)	27	48	0.0024

[Table/Fig-5]: Prevalence of abnormal Lipid Profile in ART-Naïve and ART patients

Methods

Data was collected on the socio-demographic background of each participant. HIV was diagnosed in ICTC after counseling, using 3 Test kits, HIV 1 +2 IMMUNODOT Test Kit (Enzyme Immuno Assay), HIV-1/2 Triline Card Test (Immuno chromatographic based assay) and HIV-1/2 rapid Trispot Test Kit (immune concentration based Assay).

In both the groups, 5 ml of 12 hours fasting blood sample was collected under aseptic precautions from each of the participants. This sample was used for analysis of Blood sugar, Lipid Profile {Total Cholesterol (TC), Triglycerides (TG), LDL Cholesterol (LDL-C), HDL Cholesterol (HDL-C), Non-HDL Cholesterol, VLDL and Total Cholesterol/HDL ratio}, Aspartate Transaminase. The data regarding routine investigations done at ART centre like VDRL, HbsAg, CD4 T-lymphocytes, Haemoglobin %, Total WBC & ESR was collected from the patient's case sheets.

Blood sugar and Lipid profile were analysed by enzymatic method, AST by kinetic method. LDL, Non-HDL (Total Cholesterol- HDL Cholesterol) and TC/HDL-C ratio were calculated parameters.

Statistical analysis was done using the online statistical package from the websites www.openepi.com version 3 and www.usablestats.com. A probability value of < 0.05 was taken as the threshold for statistical significance.

RESULTS

The study included 100 ART-naïve participants and 100 HIV-positive patients on ART. Of the 200 patients 34 were in Stage I (WHO Staging of HIV positive patients), 36 in Stage II, 115 in stage III and 15 in stage IV. Fifty nine HIV patients on ART were on SLN regimen (Stavudine, Lamivudine & Nevirapine) and the remaining 41 patients were on ZLN regimen (Zidovudine, Lamivudine & Nevirapine).

The average age of the ART-naïve group was 35.75 ± 9.05 years and in ART group 36 ± 8.99 years with no significant difference (p= 0.84). There was no significant difference in sex distribution. These patients were on ART for an average duration of 25.3 months with standard deviation of 13.17 months. Ten of the ART patients had Pulmonary Tuberculosis and four had tubercular meningitis. In these patients Nevirapine was substituted with Effavirenz. The weight was significantly more in ART group (48.9 + 11.11 Kgs) when compared to ART-naïve group (42.77 + 8.61 Kgs) with a p-value of 0.0001. [Table/Fig-1]. The CD4 count was significantly more in ART patients (393.66 + 160.24) when compared to ART-naïve patients (123.95 + 100.39) (p= < 0.0001).

The Blood sugar levels in both groups were within normal range. Total WBC count and ESR decreased significantly in ART patients (p= 0.0001). The AST levels were within the normal range in both the groups. In the ART-naïve group, 5 were positive for VDRL and 9 for HbsAg.

Of the 100 ART patients 23 had lipodystrophy signs and symptoms like buffalo hump [Table/Fig-2], abnormal fat deposition around neck and back, buccal fat resorption, increase in abdominal fat. Twenty one of these patients were on SLN regimen and 2 were on ZLN regimen for more than a year. Along with lipodystrophy signs and symptoms, one patient on ZLN regimen had white nail syndrome [Table/Fig-3].

There was a significant increase in serum Total Cholesterol, LDL-C, TG, VLDL, Non-HDL-C & TC/HDL-C ratio in ART patients compared to ART-naïve patients [Table/Fig-4]. The US National Cholesterol Education defines abnormal Lipid profile as TC > 200 mg/dl, HDL-C < 40 mg/dl, LDL-C > 130 mg/dl, TG > 150 mg/dl and TC/HDL-C ratio > 5 [13]. Based on this, the abnormal lipid profile was higher in ART patients compared to ART Naïve patients [Table/Fig-5]. The odds ratio (95% confidence interval, Z-statistic, p-value) in ART-Naïve patients Vs. ART patients was 0.0905 (0.0398- 0.2059, 5.728, p =<0.0001) for TC > 200mg/dl; 2.0062 (1.0444-3.8538, 2.090, p = 0.0366) for HDL Cholesterol < 40 mg/dl; 0.0570 (0.0214-0.1521, 5.722, p = <0.0001) for LDL Cholesterol >130mg/dl; 0.4007 (0.2220-0.7233, 3.035, p = 0.0024) for triglycerides >150 mg/dl and 0.1569 (0.0846-2=0.2909, 5.879, p = < 0.0001) for TC/HDL ratio 0f >5.

The lipid profiles in patients on SLN regimen (Stavudine + Lamivudine + Nevirapine) were compared to those on ZLN regimen (Zidovudine + Lamivudine + Nevirapine). The lipid profile values in the two groups were TC =194.16 ± 43.36 Vs 190.26 ± 39.72; HDL-C = 34.26 ± 9.03 Vs 35.04 ± 8.99; LDL-C = 128.27 ± 39.02 Vs 126.79 ± 37.25; Triglycerides = 158.24 ± 46.44 Vs 142.72 ± 37.76 and VLDL = 31.53 ± 8.69 Vs 28.5 ± 7.49 respectively. There was no significant difference in the two groups.

DISCUSSION

It is well known that Protease Inhibitors (PIs) induce derangements of lipid profile during ART [4,14,15]. However, evidence in support of the adverse effects of NRTI's (Zidovudine, Stavudine, Lamivudine) and NNRTI's (Nevirapine, Effavirenz) on lipid profile in HIV patients on ART is limited. Our study showed that ART patients (Zidovudine/ Stavudine + Lamivudine + Nevirapine) had significantly high levels

of Total Cholesterol, LDL-Cholesterol, Triglycerides, VLDL and high TC/HDL-C ratio as compared to ART-naïve patients. There was no significant difference in HDL-C levels between these groups. The proportion of patients with dyslipidaemia among our ART treated participants was higher than the rate reported in a study from Cameroon and rural Uganda [16,17]. There are suggestions that the magnitude of first-line ART-induced lipid derangements could vary across populations and settings. Based on LDL-C cut-off values, the prevalence rate of dyslipidemia in our study (48%) was higher than that reported in Cameroon (46.4%) [16], Western India (30%) [10] and Uganda (6%) [17]. But the prevalence of high LDL-C prior to ART (5%) was almost same as reported in Uganda and Western India (4%) [10,17], but less than that reported in Cameroon (21%) [16]. Similar to the Cameroon study [16], our study showed no changes in HDL-C levels after ART, which is not in accordance with the findings in Western India [10], which showed significant increase in HDL-C after 18 months of treatment with first-line ART regimen.

A number of studies have found that stavudine was more involved in the occurrence of lipid derangements as compared with other NRTI's [18,19]. A prospective multicentre study by the RECOVER study Group found that HIV-positive patients who replaced Stavudine with TDF(Tenofovir Disoproxil Fumarate) had significant decrease in triglycerides and cholesterol levels. This suggests, at least partly, a Stavudine (d4T)- associated dyslipidemia [7]. Our study showed no difference in lipid profiles when participants on SLN regimen (Stavudine + Lamivudine + Nevirapine) were compared to those on ZLN regimen (Zidovudine + Lamivudine + Nevirapine). These findings are similar to the findings of Buchacz et al., in Uganda [17], Pujari et al., in Western India [10] and Pefura Yone et al., in Cameroon [16].

Of the 100 patients on ART, 23 had lipodystrophy syndrome. Twenty one of these were on SLN regimen and two on ZLN regimen. The duration of NRTI therapy in the present study was 25.3 ± 13.17 months. The HIV-associated lipodystrophy syndrome was first described in 1998, shortly after the introduction of PIs [7]. It is now clear that HIV lipodystrophy can also develop in patients who have never been treated with PIs [7]. The use of NRTI's Stavudine (d4T) in particular has been linked specifically to the development of the lipoatrophic component of HIV-associated lipodystrophy syndrome [7]. There is now strong evidence that NRTI-induced mitochondrial toxicity plays a major role in the development of the lipoatrophic component of HIV-associated lipodystrophy syndrome. The NRTI's are known to have an inhibitory effect on mitochondrial DNA (mt DNA) polymerase gamma, the principal enzyme responsible for mt DNA replication. Because mt DNA encodes many of the oxidative-phosphorylation chain proteins, a decrease in mt DNA content theoretically could hinder aerobic respiration and other mitochondrial functions [6]. Improvement in both mt DNA and complex I mitochondrial enzyme activity level as well as in the rate of adipocyte apoptosis have been demonstrated following removal of the offending NRTI's [6]. Hypercortisolism has been excluded as a cause of buffalo hump (enlargement of dorsocervical fat tissue) in HIV-associated lipodystrophy and the factors associated with the development of this form of fat accumulation remain unclear [7].

In principle, dyslipidaemia associated with an increase in cholesterol and/or triglycerides suggests an increased cardiovascular risk. This suggests that treatment with first-line ART may actually have harmful effects on the cardiovascular health of the HIV patients on ART. Non-HDL Cholesterol is a better predictor of the risk of CVD than a simple measure of LDL Cholesterol [20]. Our study showed a significant increase in Non-HDL cholesterol and TC/HDL ratio in ART patients compared to ART naïve patients.

Cohort studies are needed to study the impact of dyslipidaemia on the cardiovascular health of HIV patients on ART. The Data collection on Adverse Events of Anti-HIV Drugs (D:A:D study), a prospective assessment of 23,490 patients from 11 cohorts on three continents, found that combination ART was associated with a 27% relative increase in the rate of Myocardial infarction per year of exposure during the first seven years of treatment [7].

CONCLUSION

To conclude, it is evident from our study that there is increase in lipid profile (except HDL) in ART patients compared to ART Naïve group and 23 ART patients showed lipodystrophy syndrome. Hence, it appears reasonable to measure fasting lipid levels before and 3-6 months after antiretroviral therapy is initiated or when ART regimen is changed. Whenever possible, the antiretroviral therapy least likely to worsen lipid levels should be selected for patients with dyslipidemia. The approach to ART should not only be driven to maximize the antiviral efficacy but also to reduce the current complications of the available treatments.

Scope for further Study: Decision on lipid lowering therapy can be based on estimating the 10-year risk for Myocardial infarction according to the Framingham equation (http://hin.nhlbi.nih.gov/atpii/calculator.asp). However a large prospective study is to be done to show whether this long term risk calculation is applicable for HIV infected patients given the changing lipid levels and medication regimens during HIV therapy.

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