Introduction

The availability of combined antiretroviral therapy (cART) has resulted in a dramatic decrease in opportunistic infections and mortality in human immunodeficiency virus (HIV) patients. Although the longevity of patients has increased, along with it, newer complications are also being recognized. Over the last several years, clinicians have been observing perplexing changes in fat distribution and metabolism in HIV patients, irrespective of ART.

Abstract

Background: Metabolic abnormalities are common throughout the course of human immunodeficiency virus (HIV) infection and may occur either due to HIV infection or as a result of side effects of antiretroviral therapy. It has been established that dyslipidemia and dysglycemia associated with HIV disease reduce the long-term survival of the patients, but their role for predicting prognosis of short-term mortality in HIV patients is unknown.

Aim: To study dyslipidemia and dysglycemia as a prognostic indicator for short-term mortality (<3 months) in HIV patients.

Subjects and Methods: An observational, prospective study was conducted at a tertiary care center over a period of 6 months. Consecutive HIV-positive patients hospitalized (both, HIV status known prior to hospitalization and the diagnosis made for the first time at admission) in medical wards from March to May 2010 were studied. All patients had their random blood sugars, fasting blood sugars (if possible), fasting lipid profile, and cluster of differentiation 4 (CD4) counts tested at the time of enrollment. The patients were followed for a period of 3 months, at the end of which they were categorized as survivors and non-survivors, and the demographic, clinical, and investigational parameters were compared between the above groups. Data was analyzed by applying Mann-Whitney U test, two sample t-test, Fisher-Exact test, and stepwise logistic regression analysis of significance, using the computer-based program, Stata, version 11.1.

Results: A total of 82 patients were enrolled for the study of which 64 (78.05%) were males and 18 (21.95%) were females, with a mean (SD) age of 34.00 (7.0) years. The mean CD4 count was 206.23 (129.5) cells/mm$^3$. The overall mortality within 3 months was 20.7% (17/82). Mycobacterium tuberculosis as opportunistic infection was found in 42 patients, out of which 13 expired ($P=0.02$). Patients with low high-density lipoprotein (HDL) and hypertriglyceridemia (adjusted OR = 22.92, $P$ value = 0.03, adjusted OR = 3.4, $P$ value = 0.02, respectively) had high likelihood of mortality within 3 months.

Conclusions: Low HDL and hypertriglyceridemia also appear to be promising short-term mortality markers in HIV patients apart from established factors like low CD4 counts, co-morbid conditions, and opportunistic infections like M. tuberculosi s infection. This study warrants further studies with a larger sample size to establish HDL and triglyceride as markers of disease progression and short-term mortality in HIV-infection.
C-reactive protein have shown a high correlation with all-cause short term mortality.\[^{[5]}\] Large cohort studies from Europe, North America, Asia, and Australia have shown that metabolic abnormalities are common among HIV-infected patients who receive treatment with antiretroviral drugs.\[^{[14,5]}\] Dyslipidemia is also present in HIV patients who are not on antiretroviral treatment. Hypertriglyceridemia has been described in HIV-infected patients, usually at the final stages of the disease.\[^{[6]}\] High-density lipoprotein (HDL) cholesterol level has shown a negative correlation with TNFα, TNFα receptors, and IFNγ.\[^{[7,8]}\] A low level of HDL has been observed in patients with lower cluster of differentiation 4 (CD4) counts and immune activation.\[^{[9]}\] In India, HIV patients seek medical facility in the late stages of the disease, hence are more vulnerable to short-term mortality despite the availability of antiretroviral therapy. The present study was undertaken to determine the prognostic significance of the deranged metabolic parameters (dyslipidemia and dysglycemia) for short-term mortality in HIV patients.

### Subjects and Methods

Consecutive HIV-positive patients hospitalized in medical wards at a tertiary healthcare center of north India (Gandhi Memorial and Associated Hospital, Chhatrapati Shahuji Maharaj Medical University, Lucknow) from March to May 2010 were enrolled. The total duration of the study was 6 months, during which patients were enrolled and followed up for a period of 3 months, at the end of which their outcome in terms of mortality was observed. No exclusion criteria were considered, and informed consent was obtained from all the patients/attendants. All the patients were evaluated clinically by history and examination, and relevant investigations indicated for the illness for which patient was admitted were carried out. Complete blood picture, random blood sugars, serum electrolytes, urea, creatinine, card tests for hepatitis B and C virus, and chest X-ray were done routinely in all patients. Additionally, cerebrospinal fluid examination including culture and India ink preparation for *cryptococcus*, sputum examination, stool examination, and brain radio imaging were done wherever indicated. Opportunistic infections (OIs), if present, were managed. Lipid parameters and fasting blood glucose (FBG) were determined at the time of enrollment. Levels of total cholesterol (TCH), HDL, low-density lipoprotein (LDL), triglyceride (TG), and fasting serum glucose were measured by using standard enzymatic techniques. Plasma glucose was estimated by O-Toluidine method. TG and HDL and TCH were measured by GPO-POD method, PEG Precipitation method, and CHO-POD method, respectively. LDL and very low-density lipoprotein were estimated indirectly by Friedewald equation, i.e., LDL cholesterol = TCH-[HDL Cholesterol + TG / 5].

The following definitions were used for dyslipidemia:

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TCH \geq 200 \text{ mg/dL}, \quad LDL \geq 150 \text{ mg/dL}, \quad TG \geq 170 \text{ mg/dL}, \quad \text{and} \quad \text{HDL cholesterol} < 35 \text{ mg/dL}. 
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In addition, hyperglycemia was defined as a FBG concentration \(\geq 100 \text{ mg/dL}\). All patients had absolute CD4 lymphocyte counts done by flow-cytometry and defined as cells/mm\(^3\). No control group was taken for the study. Rather, at the end of the 3 months’ follow-up, patients were categorized into two groups, i.e., survivors and non-survivors. The baseline and investigational characteristics were compared between these groups.

### Statistical analysis

Data was presented either as mean (SD) or as percentage and median. A comparison between survivors and non-survivors was made by Mann-Whitney U test/Two sample ‘t-tests’ for quantitative data and Fisher Exact test for categorical data. A stepwise logistic regression analysis was applied to identify the independent association of diverse parameters with short-term mortality. The factors which were associated with outcome at \(P < 0.25\) were considered for logistic regression analysis. A factor influencing outcome of interest was considered significant if its \(P\) value was \(<0.05\). All analyses were performed with the statistical software Stata, version 11.1 (StatCorp Inc., College Station, Texas, USA). The study was approved by the ethics committee of our institute.

### Results

A total of 82 HIV-positive patients were studied. The mean age of the patients was 34 (7) years. Males were 78.0% (64/82) and females were 22.0% (18/82). OIs were present in 52 (63.4%) patients out of whom 42 (80.7%) patients had *Mycobacterium tuberculosis* (two patients of pulmonary tuberculosis, 40 patients of extrapulmonary tuberculosis). Cryptococcosis was present in five patients, *Pneumocystis jiroveci* pneumonia in three patients, toxoplasmosis in two patients, and candida infection was seen in eight patients. Hepatitis B co-infection was also present in four patients. Of the patients, 56 (68.3%) were in WHO stage IV (classified as acquired immunodeficiency syndrome/AIDS) and 26 (31.7%) were in WHO stages I-III (non-AIDS). Antiretroviral therapy was being used by 31 (37%) patients according to guidelines of National AIDS Control Organization. The median CD4 lymphocytes count was 193/mm\(^3\) (IQR 102–271/mm\(^3\)). Dyslipidemia was seen in 81 (98.7%) patients, in the form of low HDL, raised TG, or both. Low HDL was the predominant dyslipidemia, followed by hypertriglyceridemia, raised TCH (\(\geq 200\) mg/dL), and raised LDL; found in 69.5% (57/82), 30.5% (25/82), 8.5% (7/82), and 6.1% (5/82) of the patients, respectively. In Table 1, the descriptive statistics of the studied population is shown.

At the end of the study, 17 (20.7%) patients did not survive. Demographic and laboratory parameters were compared between the survivors and non-survivors as shown in Table 1.

Among the survivors, the mean age was 34.0 (6) years, whereas it was 30.0 (6) years in non-survivors \(P=0.014\). The mean hemoglobin level in survivors was 8.38 (3) g/dL, whereas in non-survivors it was 9.2 (1.7) g/dL. Between the survivors and
non-survivors, the only metabolic parameter which showed significant difference was the mean TG level (134.6 (37.6) vs. 191.9 (75.3), P value <0.001) while the means of fasting blood sugar (FBS), TCH, HDL, and LDL varied insignificantly [Table 2]. The multivariable analysis showed that the variables; *M. tuberculosis* infection, the stage of disease (dichotomized as non-AIDS and AIDS categories), CD4 counts (categorized in <200/mm³, 200–350/mm³ and >350/mm³), FBS (<100 mg/dL and >100 mg/dL), hypertriglyceridemia (>170 mg/dL), and low HDL (<35 mg/dL) were found to be statistically associated with mortality at P < 0.25 level. In order to remove the confounders or to adjust them, if removal was not possible, logistic regression analysis was done for all the above predictors, thus negotiating the effect of confounders. Out of 82 patients, only 46 patients were retained in the model (FBS was not available in 28 subjects and all eight subjects with CD4 count >350/mm³ survived, resulting in drop out of 36 patients). Maximum likelihood estimation model was used applying “enter method” to fit the best model and efforts were made to keep those variables, which contributed to the model [Table 3]. Patients with low HDL (adjusted OR=22.92, P value=0.032) had a higher likelihood of mortality. However, the deranged FBS and presence of *M. tuberculosis* infection had higher risk for mortality, and higher CD4 count (>200/mm³) reduced the mortality by 21%, though these were not statistically significant.

### Discussion

The results of our study suggest that low HDL and hypertriglyceridemia were more commonly seen in HIV patients who died within a period of 3 months’ follow-up. The typical patterns of dyslipidemia seen in the HIV patients were low level of HDL, elevated TG, and raised TCH levels. In the study, besides low HDL and hypertriglyceridemia, *M. tuberculosis* infection and low CD4 counts were the other variables associated with early mortality. The lipid abnormalities may be associated with insulin resistance and glucose intolerance in HIV patients.[10] Evidence has established immune activation as a critical underlying mediator of immune dysfunction and immune deficiency in HIV disease.[9,11,12] This state of immune activation is manifested both by enhanced expression of phenotypic activation markers on peripheral blood T cells and B cells and by increased plasma
levels of inflammatory cytokines. Among the cytokines, the most consistent and potent inducers of HIV expressions are the pro-inflammatory cytokines: TNFα, IL-1, and IL-6.\(^{[13]}\) Dyslipidemia in HIV-infected/AIDS patients may occur even during the early stages of HIV infection and, more so as the disease progresses.\(^{[14]}\) Decrease in TCH and HDL cholesterol has been reported in the early stages of HIV infection, being more evident with decreasing CD4+ lymphocyte counts.\(^{[6]}\) Recently, efforts have been made to correlate acute phase reaction (APR) and lipid metabolism.\(^{[15]}\) Low HDL is associated with the presence of high plasma levels of IL-6, independent of the influence of a large number of possible confounders, including the main traits of the metabolic syndrome (triglycerides, fasting insulin, diabetes, hypertension, BMI, waist circumference), and lifestyle habits (smoking, alcohol intake, physical activity). The above mentioned conditions are frequently associated with significant modifications in both HDL and IL-6 plasma level.\(^{[16,17]}\) Interleukin 6, together with other cytokines, might influence HDL levels by modifying the activity of the triglyceride lipases. It has been shown that pro-inflammatory cytokines inhibit the activity of lipoprotein lipase\(^{[18]}\) and enhance the lipolytic activity of endothelial lipase.\(^{[19,20]}\) Both these actions have been associated with low HDL levels during acute or chronic inflammatory states. Unlike normal HDL particles, high-density lipoproteins modified by APR do not exhibit any in vitro anti-inflammatory property. However, they are converted to pro-inflammatory molecules, perhaps as a consequence of their increased ceruloplasmin content.\(^{[21]}\) Dyslipidemia is also attributed to the use of non-nucleoside, nucleoside reverse transcriptase inhibitors, and protease inhibitors. Newer drugs, Raltegravir and Maraviroc, have proven not to be detrimental to the lipid profile.\(^{[22,23]}\)

In developing countries, patients report late, often in advanced stages of the disease, with a low CD4 count and a number of complications, including metabolic ones. We have observed that among the non-survivors the frequency of advanced stages of disease (AIDS) and OIs, particularly, M. tuberculosis infection, and hypertriglyceridemia were significantly higher than survivors. Though the frequency of low HDL was higher in non-survivors, it was not significant. This might have attributed to the effect of other variables affecting the outcome in concert. Adjusted results showed that low HDL was an independent strong factor (P<0.02) influencing the outcome. Surprisingly, in this study, low CD4 count (<200/mm\(^3\)) was not a strong predictor for short-term mortality. The reason would probably be the short follow-up period. Anemia has been widely reported as the time of diagnosis, which has been shown as a strong independent predictor of survival in HIV patients.\(^{[24]}\) Though the frequency of low BMI (<17.5 kg/m\(^2\)) was higher among non-survivors than survivors, it was also insignificant in our patients. The reason would probably be the short follow-up period.

Our study had some limitations such as the number of subjects was small, only hospitalized patients, who are usually very sick and have poor survival chances, were observed, only single time evaluation of lipid parameters was done, and all parameters could not be tested in all the patients. Moreover, the cause of mortality was not taken into account. In addition, no control group was considered.

The pathophysiology by which deranged metabolic parameters influence the poor short-term outcome in HIV disease, independent of other factors, is largely unknown. The probable mechanism being that in the presence of high IL-6 levels, HDL particles might be modified into pro-inflammatory molecules and thus maintain the inflammatory process in a kind of vicious circle. Advanced stages of the disease and heightened acute or chronic inflammation may be some of the contributing factors. This undertaking may have an important implication that in resource-poor settings, CD4 counts, HIV viral load measurements, and established nonspecific serological markers of inflammation and/or coagulation such as IL-6, D—dimer, and high-sensitivity C-reactive protein are not always in place: Therefore, an algorithm using less sophisticated markers, such as serum HDL and TG levels, may be a useful adjunct for assessing disease progression and informing treatment and care.

Though the study has a few shortcomings, we would like to conclude that low HDL and hypertriglyceridemia are associated with increased short-term mortality in HIV disease, and it reinforces the notion that all patients who are being hospitalized should have a baseline evaluation of the said lipid parameters as well as should be monitored regularly, which would help in predicting their short-term mortality. Ours study was a pilot study; further work is needed, which would establish them as prognostic markers as well as markers of disease progression.

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References

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