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Full Length Research Paper

Antiretroviral therapy and genetic predisposition: Cofactors contributing to the lipodystrophy syndrome

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The TNF-α-238G/A single nucleotide polymorphism (SNP) independently increases the risk of lipodystrophy progression. The current study evaluates the contribution of the different antiretroviral treatments (ART) regimens administered to HIV infected individuals in Pune to the development of lipodystrophy and insulin resistance in patients having TNF-α-238G/A polymorphism. A total of 172 HIV-1 infected patients were enrolled in the study and were subdivided into patients with [HIV+LIPODYS+ (cases)] and without lipodystrophy [HIV+LIPODYS- (controls)]. We assessed the contribution of the typical 2NRTI+1 NNRTI based ART regimens prescribed in our clinics with the development of dyslipidemia and insulin resistance in both the above study group. A significantly higher percentage of patients with lipodystrophy expressed the TNF-α-238G/A polymorphism. Significant correlations were observed between Adiponectin, TNF-α protein levels and type of ART-ART regimen. Our results showed that the NNRTI efavirenz contributed more to the lipodystrophy syndrome as compared to other antiretroviral drugs tested. The TNF-α 238G/A SNP contributes to changes in insulin sensitivity and lipodystrophy.

Key words: HIV-1, antiretroviral drugs, single nucleotide polymorphism, lipodystrophy, dyslipidemia, adiponectin, insulin resistance, metabolic syndrome.

INTRODUCTION

Antiretroviral therapy (ART) that includes HIV-1 protease inhibitors (PIs) is considered be an important cause of peripheral lipoatrophy, dyslipidemia, lipodystrophy, and insulin resistance [John et al (2001); Nolan (2003); Brown and Cofrancesco (2006); Grunfeld et al., (1989); Caron-Debarle et al (2010)]. However, lipodystrophy can also occur in the absence of PI's as well, and is mostly associated with non-nucleoside reverse transcriptase inhibitors (NNRTI) efavirenz (EFV). Lipoatrophy however is mostly associated with thymidine analogue nucleoside reverse transcriptase inhibitor (NRTI), (d4T > zidovudine (ZDV) [Caron-Debarle et al., (2010); et al., 2011; Swaminathan et al., (2010) Parakh et al., (2009); Patel et al (2006); Gupta et al., (2006)]. Under the national free

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ART program in India, the eligible HIV-1 patients receive 2NRTI+1NNRTI based regimen. Of those who receive first-line ART, about 60% were receiving stavudine-based regimen. Stavudine is being phased out from the free ART program in India. Efavirenz based regimen is offered to those who have tuberculosis, a common opportunistic infection in Indian patients. Once the patient fails on first line ART, the program offers them a PI-based regimen. Both efavirenz and stavudine are known for impacting lipid levels adversely (Vigano et al., 2005; Jones et al., 2005).

A few studies have also revealed that the incidence of chronic cardiac morbidities including myocardial infarction increases among those who are receiving antiretroviral therapy based on certain antiretrovirals such as stavudine, efavirenz, ddI, abacavir and certain protease inhibitor drugs (Barbaro, 2003); Boccara, 2008; Villarroya et al., 2009). In a recent study in India, HIV-1 associated lipodystrophy was documented in 45.5% of patients (Pujari, 1998; Pujari et al., 2005; 2004). The study also reported increased prevalence of dyslipidemia and hyperglycemia in patients with lipodystrophy who were on long term ART therapy given that there is a high prevalence of dyslipidemia among Indians taking long-term PI's (Pujari et al., 2005; 2004).

Variations in host genetic susceptibility, as manifested by single nucleotide polymorphism (SNP) allelic variants of TNF-α are likely to influence development of lipo-dystrophy (Marzocchetti et al., 2011; Maher et al., 2002) via the modulation of TNF-α expression. TNF-α-238G/A promoter polymorphism independently increases the risk of lipodystrophy progression in white Caucasian ART recipients patient cohorts (Maher et al., 2002; Campbell et al., 2012; Mehendale et al., 2002; Mondal et al., 2001; Aboud et al., 2007; Hadigan et al., 2001; Kino et al., 2003a; 2003b; Lanzafame et al., 2003; Sethi and Homtamisligil, 1999; Domingo et al., 2005).

This is a controversial issue as some investigators have shown that there is an association between TNF-α alleles and HIV lipodystrophy and that certain TNF-α SNPs confer protection while others made them more susceptible to lipodystrophy progression. Maher et al. (2002) have shown that the -238 promoter region TNF-alpha gene polymorphism is a significant determinant in the development of HIV-related lipodystrophy (Maher et al., 2002).

However, on the other hand Veloso et al. (2011) showed that there is a systemic overproduction of soluble TNF-α R2, in HIV-1 subjects on ART and that this is not related to lipodystrophy in these individuals. Their meta-analysis studies, also showed no associations between the TNF-α -238G > A and -308G > A SNP and lipodystrophy[31]. Given the controversy in literature (Maher et al., 2002; Campbell et al., 2012; Mehendale et al., 2002; Mondal et al., 2001; Aboud et al., 2007; Hadigan et al., 2001; Kino et al., 2003a; 2003b; Lanzafame et al., 2003; Sethi and Homtamisligil 1999; Domingo et al., 2005; Veloso et al., 2011) a link between the TNF-α -238G > A SNP and lipodystrophy, additional larger and ethnically diverse patient populations need to be studied. In India, although the prevalence of the genetic polymorphism in the TNF-α - 238 G>A in the general control population was reported to be about ~ 30%, and significant differences in the prevalence of this SNP is reported between the ethnically different North Indian (Indo European or Caucasoid origin) vs South Indian (Dravidian origin) groups (Singh et al., 2011). However, there is no data on its prevalence among persons living in Western parts of India which is predominantly our patient cohort. We determined the contribution of TNF-α-238G/A SNP in the development of HIV-1 lipodystrophy among our HIV infected patient cohorts who have been receiving ART in the immunodeficiency clinics of the National AIDS research Institute (NARI) at Pune, India. Additionally, we assessed the contribution of the typical 2NRTI+1NNRTI based ART regimens with the development of HIV-1 lipodystrophy and dyslipidemia in patients with the TNF-α-238G/A polymorphism. This investigation may provide insight into the role of antiretroviral therapy among HIV-1 infected individuals in patients with TNF-α SNP and its increased expression.

METHODOLOGY

Study design

A total of 184 HIV-1 patients were enrolled from 3 separate NARI clinics in different parts of Pune, a city located in Western India. The study was approved by the Institutional review board (IRB). Informed consent was obtained for all patients and patients were de-identified and encrypted to prevent individual identification and to provide utmost privacy consistent with HIPPA (U.S. Health Insurance Portability and Accountability Act of 1996) guidelines.

Inclusion/exclusion criteria

The following were the inclusion criteria for the study:

1. HIV-1 infected adults receiving antiretroviral therapy for at least one year irrespective of whether or not they expressed the TNF-α -238 G>A SNP.
2. Patient's willingness to participate in the study. The exclusion Criteria included Any HIV-1 infected individual with concurrent conditions such as Tuberculosis, Hepatitis B/C, autoimmune diseases, Cancers, Diabetes Mellitus, and past history of cardiovascular disease; Patients who consume alcohol every day.
3. Patients who used recreational drugs. Subjects in both groups were age (within +/-3 years) and gender matched.

Definitions

Lipodystrophy: HIV-1 patients with clinical presentation of HIV associated lipoatrophy/lipohypertrophy and dyslipidemia.

Dyslipidemia: HIV-1 patients with dyslipidemia were defined by

performing lipid profile after 14h of fasting as: Cholesterol >240 mg and/ or Triglyceride > 300 mg/dL and/or LDL >120 mg/dL and/or; decreased level of HDL <35 mg/dL. (These cutoff values are validated and adopted by the NARI Clinical Pathology laboratories which are relevant to our patient population).

Impaired fasting glucose: Was defined as fasting plasma glucose > 100 mg/dL. The homeostatic model assessment (HOMA) was used to quantify insulin resistance and beta-cell function.

Insulin resistance (IR): IR was defined by the homeostasis model assessment of insulin resistance (HOMA−IR index) and β cell function (HOMA B index). These indexes were calculated using the following formulas, where insulin levels are in mu/mL and glucose levels are converted to molar units mmol/L.

HOMA-IR index =(fasting insulin x fasting glucose)/22.5; HOMA B index = [20x insulin (mU/mL)]/[glucose (mmol/L)−3.5 IR was defined when the value of HOMA >4 [Matthews et al (1985)]. Of the 184 patients enrolled only 172 patients were classified into 2 study groups outlined below. 12 patients were excluded due to incomplete data. HIV+LIPODYS+ (cases) (n=101): HIV-1 positive patients with lipodystrophy. These patients had abnormal fat distribution as observed in lipohypertrophy or lipoatrophy,or a combined phenotype of both lipohypertrophy and lipoatrophy, along with dyslipidemia.

Lipoatrophy: Lipoatrophy was defined as fat loss in ≥1 of the following sites: face (sunken cheeks/eyes, or prominent zygomatic arch), arms and legs: (skinny with prominent veins, muscularity or bones), and buttocks (loose skin folds, prominent muscles, or loss of contour and fat, or hollowing).

Lipohypertrophy: Lipohypertrophy was defined as fat gain in ≥1 of the following sites: trunk (increased abdominal girth), base of neck/back (“buffalo hump”), or breast enlargement. These patients also had significant dyslipidemia. HIV+LIPODYS- (controls) (n=71): HIV-1 positive patients with no HIV associated lipodystrophy and/or dyslipidemia. Clinical parameters such as blood glucose, lipid profile (Cholesterol, Triglycerides, HDL and LDL) were done using COBAS Integra 800 analyzer (Roche1Diagnostics Ltd, Indianapolis, IN) and the CD4 counts were obtained by FACS analysis, all these parameters are tested as part of routine clinical investigations during regular clinic visits by the patients. Levels of TNF-α, Adiponectin and Insulin in patients enrolled in the study were determined using commercially available ELISA kits from RayBiotech Inc, Norcross, GA. The past history about antiretroviral therapy was recorded. Antiretroviral therapy (ARTART) based on World Health Organization recommended pre-qualified fixed dose combinations of at least three ART drugs including ≥1 NRTI, with ≥1 NNRTI or ≥1 PI. The typical ART drug combination used at NARI clinics are stavudine/lamivudine/nevirapine (d4T/3TC/NVP) (SLN) or stavudine/lamivudine/efavirenz (SLE) or zidovudine/ lamivudine/ nevirapine (d4T/3TC/NVP)(ZLN) or zidovudine/lamivudine/ efavirenz (ZLE) (Padmapriyadarshini et al., 2011; Patel et al., 2006; Gupta et al., 2006; Campbell et al., 2012; Mehendale et al., 2002).

Statistically analyses

Data analysis was based on comparisons between cases and controls for all parameters tested. A one way ANOVA test was done to determine interactions between two factors and the two–way ANOVA/mixed model analysis of variance was done for multiple comparisons followed by post hoc tests (Bonferroni) to determine significance between the 4 ART groups. Group (A) SLE [stavudine (d4T)/lamivudine (3TC)/efavirenz (NNRTI)]; Group (B) ZLE [zidovudine (NRTI)/lamivudine (3TC)/efavirenz]; Group (C) SLN [stavudine (d4T)/lamivudine (3TC)/nevirapine (NVP) (NNRTI)]; Group (D) ZLN (zidovudine/lamivudine (3TC)/nevirapine (NVP)]. To evaluate which clinical parameter is most significantly affected by the type of ART in patients with HIV associated lipodystrophy, additional correlation analysis was done in this patient group comparing different ART paradigms. The correlation coefficient was determined using Pearsons correlation analysis for all the parameters tested. A p-value < 0.05 was considered to be significant. The statistical software package GraphPad Prism (GraphPad Prism Software, Inc. San Diego, CA) was used to analyze this data. Odds Ratio was calculated using the odds ratio calculator (http://www.hutchon.net/ConfidOR.htm (Bland and Altman, 2000).

RESULTS

Table 1 shows the average number of years on ART, average age, body mass index (BMI) and CD4 count for the patients in the four ART groups. No significant differences were observed in any of these parameters in the four ART groups. Majority of the patients at the NARI clinics were on SLN or ZLE regimens. Fewer patients, 3% on SLE and 11% on ZLE were on Efavirenz, since it was significantly associated with the development of lipodystrophy. Of the total of 172 HIV-infected patients who were receiving antiretroviral therapy for at least one year, 101 patients had HIV-1 associated lipodystrophy (cases), while 71 patients did not have lipodystrophy (controls). There were no significant differences in time duration since onset of infection between the 2 study groups. Distribution of the percentage of patients with lipodystrophy in the four ART groups was as follows; 80% of patients on an SLE regimen had lipodystrophy (4), as compared to 61% (11), 63% (47), and 54% (39) in patients on ZLE, SLN and ZLN regimens, respectively. Data was analyzed to determine which antiretroviral drug contributed the most to the development of HIV lipodystrophy and our results showed that 71% of patients on Efavirenz developed lipodystrophy as compared to 66% on Stavudine, 60% on Nevirapine and 57% on Zidovudine respectively, suggesting that Efavirenz treatment contributed most to the development of the lipodystrophy syndrome as compared to the use of other antiretroviral drug. When comparisons were made between patients with and without lipodystrophy with respect to the lipid profile parameters, no significant differences in cholesterol or LDL levels were observed between the ART groups, while triglycerides levels were consistently higher in patients with lipodystrophy across all the four ART groups. HDL levels too were significantly lower in patients with lipodystrophy across all the four ART groups, indicating that triglycerides and HDL were more likely to be associated with lipodystrophy as shown in Table 2. Table 3 shows the metabolic parameters measured in the patients with and without lipodystrophy in the 4 ART groups. We did not observe any significant differences in blood glucose levels, however, as expected, the insulin levels and the insulin resistance as measured by HOMA were higher in the patients with lipodystrophy in all the four ART

Table 1. Patient characteristics in the four ART groups.

Characteristic	HIV+LIPODYS-	HIV+LIPODYS+	P value
No. of years on ART			
Group A=SLE	4.06 ± 2.05 (n=3)	6.83 ± 5.10 (n=4)	NS
Group B=ZLE	8.20 ± 4.15 (n=7)	8.43 ± 4.43 (n=11)	NS
Group C=SLN	5.38 ± 3.50 (n=28)	6.92 ± 4.08 (n=47)	NS
Group D=ZLN	7.25 ± 3.42 (n=33)	7.08 ± 4.57 (n=39)	NS
Age (years)			
Group A=SLE	35.01 ± 2.02 (n=3)	35.75 ± 4.50 (n=4)	NS
Group B=ZLE	37.80± 4.32 (n=7)	38.77 ± 5.90 (n=11)	NS
Group C=SLN	36.81 ± 7.69 (n=28)	37.91 ± 7.12 (n=47)	NS
Group D=ZLN	38.06 ± 7.28 (n=33)	38.74 ± 7.11 (n=39)	NS
Body mass index (BMI)			
Group A=SLE	19.00 ± 2.12 (n=3)	20.02 ± 2.94(n=4)	NS
Group B=ZLE	22.24± 3.03 (n=7)	20.95 ± 3.89(n-11)	NS
Group C=SLN	20.02± 2.29 (n=28)	21.25 ± 3.72(n=47)	NS
Group D=ZLN	20.76 ± 3.18 (n=33)	21.47 ± 3.57(n=39)	NS
CD4 counts			
Group A=SLE	432.0 ± 105.1 (n=3)	494.75 ± 122.63(n=4)	NS
Group B=ZLE	467.40 ± 207.20 (n=7)	627.55 ± 261.02(n=11)	NS
Group C=SLN	600.26 ± 231.46 (n=28)	554.13 ± 222.39(n=47)	NS
Group D=ZLN	569.18 ± 239.31 (n=33)	478.29 ± 238.99(n=39)	NS

Data is expressed as mean ± standard deviation and statistical significance is calculated based on the difference in mean value between HIV positive patients with and without lipodystrophy (HIV+LIPODYS- vs HIV+LIPODYS+) divided into four groups based on their ART regimen (Group A to D). A p value of < 0.05 is considered statistically significant; NS = No significance. The description of the ART groups is as follows: Group A (SLE) consists of (n=7) patients receiving Stavudine, Lamivudine and Efavirenz; Group B (ZLE) consists of (n=18) patients receiving Zidovudine, Lamivudine and Efavirenz; Group C (SLN) consists of (n=75) patients receiving Stavudine, Lamivudine and Nevirapine and Group D (ZLN) consists of (n=72) patients receiving Zidovudine, Lamivudine and Nevirapine.

groups.

Patients with HIV lipodystrophy who were on SLN and ZLN ART regimens had significantly higher insulin resistance. Although, significant differences were observed in the adiponectin levels as shown in Figure I, there were no significant differences in the TNF-α plasma levels (Table 3) in the 4 ART groups. Figure 1 outlines the differences in plasma adiponectin levels between the $HIV^{+}LIPODYS^{+}$ vs. HIVLIPODYS in the 4 ART groups. Significant differences were observed in Adiponectin levels (pg/ml) between the patients with and without lipodystrophy within the 4 ART groups, wherein lower Adiponectin levels were found in the SLE (1.80±0.21 vs. 0.94±0.6; p=0.05) and ZLE (1.97±0.2 vs 1.84±0.6; p=0.04) groups respectively. Marginally higher levels of Adiponectin were observed in the SLN (1.55±0.62 vs. 1.66±0.4; p=0.01) and ZLN (1.50±0.5 vs. 1.60±0.4; p=0.01) groups respectively. We did a correlation analysis between the different ART groups for all the parameters tested in the HIV lipodystrophy patient group and observed statistically significant correlations only with respect to Adiponectin levels in patients on SLE (OR=1.22,95% CI 0.99-1.31,p=0.01), ZLE (OR=1.09,95% CI 0.93-1.17,p=0.04), SLN (OR=1.06, 95% CI 0.89-1.13,p=0.05), and ZLN (OR=1.25, 95% CI 1.17-1.39, p=0.01) indicating that adiponectin levels was independent risk factor for lipodystrophy with all the 4 ART regimens. We observed an increase in the expression of TNF-α 238G/A SNP in patients with lipodystrophy (cases) as compared to patients without lipodystrophy (controls) in all the 4 ART regimen groups (Table 4). TNF-α 238G/A SNP expression was 6.33 (p<0.001), 3.53 (p<0.001), 1.63 (p<0.01) and 1.23 (p<0.05) fold higher in ZLE, >SLN>ZLN>SLE, ART treatment groups respectively.

Discussion

The use of ART significantly reduces the incidence of most AIDS defining illness and associated mortality (Campbell et al., 2012; Mehendale et al., 2002; Mondal et al., 2001; Aboud et al., 2007; Hadigan et al., 2001). The goal of any antiretroviral regimen is to achieve

Table 2. Patient lipid profile in the four ART groups.

Lipid profile	HIV+LIPODYS-	HIV+LIPODYS+	P value
Cholesterol (mg/dl)			
Group A=SLE	185.32 ± 51.2 (n=3)	195.67 ± 76.02 (n=4)	NS
Group B=ZLE	179.90 ± 51.15 (n=7)	181.03 ± 54.56 (n=11)	NS
Group C=SLN	179.72 ± 39.14 (n=28)	183.63 ± 50.26 (n=47)	NS
Group D=ZLN	172.46 ± 33.13 (n=33)	173.08 ± 47.82 (n=39)	NS
HDL (mg/dl)			
Group A=SLE	74.891 ± 10.02 (n=3)	39.10 ± 9.72 (n=4)	0.05
Group B=ZLE	59.15 ± 21.38 (n=7)	41.33 ± 11.53 (n=11)	0.03
Group C=SLN	64.60 ± 19.62 (n=28)	37.06 ± 11.32 (n=47)	0.001
Group D=ZLN	58.82 ± 8.49 (n=33)	39.77 ± 8.67 (n=39)	0.001
LDL (mg/dl)			
Group A=SLE	111.36 ± 41.2 (n=3)	126.02 ± 69.38 (n=4)	NS
Group B=ZLE	122.56 ± 35.03(n=7)	127.11 ± 43.62 (n=11)	NS
Group C=SLN	113.78 ± 46.03 (n=28)	113.08 ± 33.47 (n=47)	NS
Group D=ZLN	110.86 ± 28.87 (n=33)	113.62 ± 43.07 (n=39)	NS
Triglycerides (mg/dl)			
Group A=SLE	59.07 ± 22.05 (n=3)	216.39 ± 123.7 (n=4)	0.01
Group B=ZLE	68.97 ± 12.78 (n=7)	156.33 ± 53.02 (n=11)	0.001
Group C=SLN	102.88 ± 54.17 (n=28)	260.32 ± 303.38 (n=47)	0.01
Group D=ZLN	86.62 ± 47.15 (n=33)	188.41 ± 106.52 (n=39)	0.001

Data is expressed as mean ± standard deviation and statistical significance is calculated based on the difference in mean value between HIV positive patients with and without lipodystrophy (HIV+LIPODYS- vs HIV+LIPODYS+) divided into four groups based on their ART regimen (Group A-D). A p value of <0.05 is considered statistically significant; NS = No significance. The description of the ART groups is as follows: Group A (SLE) consists of (n=7) patients receiving Stavudine, Lamivudine and Efavirenz; Group B (ZLE) consists of (n=18) patients receiving Zidovudine, Lamivudine and Efavirenz; Group C (SLN) consists of (n=75) patients receiving Stavudine, Lamivudine and Nevirapine and Group D (ZLN) consists of (n=72) patients on a cocktail of Zidovudine, Lamivudine and Nevirapine.

maximal and durable virologic suppression to preserve immunologic function, and to reduce morbidity/mortality, associated with HIV infection. However, ART is associated with certain adverse effects such as lipodystrophy (Campbell et al., 2012; Mehendale et al., 2002; Mondal et al., 2001; Aboud et al., 2007; Hadigan et al., 2001). However, not all patients who receive antiretroviral drugs develop HIV-1 associated lipodystrophy, therefore a host genetic predisposition may predispose certain individuals to development of the lipodystrophy syndrome (Kino et al., 2003a, b). In western literature, it is established that the 238-promoter region TNF-α gene polymorphism is a determinant in the development of HIV-related lipodystrophy (Sethi and Hotamisligil, 1999; Domingo et al., 2005). We re-cently examined the expression of the TNFalpha-238G/A polymorphism in our present Western Indian patient cohort and wanted to report the effect of the current ART drug regimens on the development of lipodystrophy in this patient cohort. The goal of the current study was to evaluate the association between the type of antiretroviral therapy and how it contributes to the lipodystrophy syndrome via the modulation of key metabolites that are known to be involved in the pathophysiology of lipodystrophy. Our data indicates that efavirenz treatment contributes more to the lipodystrophy syndrome as compared to other antiretroviral drugs such as stavudine, nevirapine and zidovudine.

Table 1 which shows no significant differences in average number of years of ART, average age, CD4 counts and body mass index (BMI) in the patients among the four antiretroviral treatment regimen groups suggests that any difference we observed in metabolic profile may be primarily attributed to differences in the type of ART regimen. Patients enrolled in this study typically receive ART which includes a 2NRTI+1NNRTI based regimen. Majority of the patients in our study were either on ZLN or SLN treatment regimens. Efavirenz and stavudine are both known to result in dyslipidemia by altering its differentiation and insulin

Table 3. Metabolic parameters in the four ART groups.

Metabolic parameter	HIV+LIPODYS-	HIV+LIPODYS+	P-value
Insulin uIU/ml			
Group A=SLE	5.90 ± 1.11 (n=3)	11.40 ± 7.66 (n=4)	0.05
Group B=ZLE	9.86 ± 5.29(n=7)	18.81 ± 19.38 (n=11)	NS
Group C=SLN	13.47 ± 9.03(n=28)	18.87 ± 16.45 (n=47)	0.01
Group D=ZLN	12.03 ± 7.52 (n=33)	19.92 ± 13.38 (n=39)	NS
HOMA-IR			
Group A=SLE	1.03 ± 1.12 (n=3)	2.10 ±1.47 (n=4)	NS
Group B=ZLE	2.20 ± 1.80(n=7)	3.03 ± 3.74(n=11)	NS
Group C=SLN	2.46 ± 1.63(n=28)	3.64 ± 3.66 (n=47)	0.001
Group D=ZLN	2.27 ± 1.39 (n=33)	4.40 ±3.88 (n=39)	0.001
TNF-α pg/ml			
Group A=SLE	83.12 ± 45.12 (n=3)	91.1 ± 34.88 (n=4)	NS
Group B=ZLE	142.51 ± 111.16 (n=7)	101.26 ± 69.01 (n=11)	NS
Group C=SLN	72.32 ± 34.53 (n=28)	79.33 ± 20.57 (n=47)	NS
Group D=ZLN	76.29 ± 31.73 (n=33)	81.26 ± 31.59 (n-39)	NS

Data is expressed as mean ± standard deviation and statistical significance is calculated based on the difference in mean value between HIV positive patients with and without lipodystrophy (HIV+LIPODYS- vs. HIV+LIPODYS+) divided into four groups based on their ART regimen (Group A-D). A p-value of <0.05 is considered statistically significant; NS = No significance. The description of the ART groups is as follows: Group A (SLE) consists of (n=7) patients receiving Stavudine, Lamivudine and Efavirenz; Group B (ZLE) consists of (n=18) patients receiving Zidovudine, Lamivudine and Efavirenz; Group C (SLN) consists of (n=75) patients receiving Stavudine, Lamivudine and Nevirapine and Group D (ZLN) consists of (n=72) patients on a cocktail of Zidovudine, Lamivudine and Nevirapine.

Table 4. TNF-α-238G/A SNP gene expression levels in the 4 ART groups.

TNF-α-238G/A SNP gene expression expressed as transcript accumulation index or TAI=2^{-ddCT}	HIV+LIPODYS- (n=71)	HIV+LIPODYS+ (n=101)	P value
	TAI=2^{-ddCT}	TAI=2^{-ddCT}	
Group A=SLE	0.52 (n=3)	0.64 (n=4)	0.05
Group B=ZLE	0.06 (n=7)	0.38 (n=11)	0.001
Group C=SLN	0.15 (n=28)	0.53 (n=47)	0.001
Group D=ZLN	0.28 (n=33)	0.47 (n=39)	0.01

Data is expressed as mean and statistical significance is calculated based on the difference in mean value between the HIV+LIPODYS- vs. HIV+LIPODYS+ groups divided into four groups based on their ART regimen (Group A-D). A p value of <0.05 is considered statistically significant. The TNF-α-238G/A SNP gene expression levels were measured by quantitative real time PCR using primers specific to the SNP and results are expressed as transcript accumulation index or TAI. Quantitative real-time PCR (Q-RT-PCR) was used to quantitate the relative abundance of the SNP using specific primers and a Q-PCR assay kit (Cat #s 4309155 & 4306736) from Applied Biosystems (Life Technologies; Grand Island, NY) using standard protocols provided by the manufacturer.

sensitivity and also the pattern of secretion of adiponectin by adipose tissue (Vigano et al., 2005; Jones et al., 2005). The pathogenesis of ART-associated lipodystrophy and metabolic syndrome is complex and a number of factors are involved, including direct effects of ART on lipid metabolism and adipocyte cell function (Pujari, 1998; Veloso et al., 2011; Sevastianova et al., 2008; Brown and Confrancesco, 2006; Stankov and Behrens, 2010). Fat redistribution may precede the development of metabolic complications associated with the lipodystrophy syndrome (Sevastianova et al., 2008; Brown and Confrancesco, 2006; Stankov and Behrens, 2010). Table 2 shows that levels of triglycerides and HDL were significantly different between the patients with and without lipodystrophy. We did not observe significant differences in cholesterol and LDL plasma levels between HIV-1-infected patients with and without lipodystrophy, this may be attributed to the fact that although

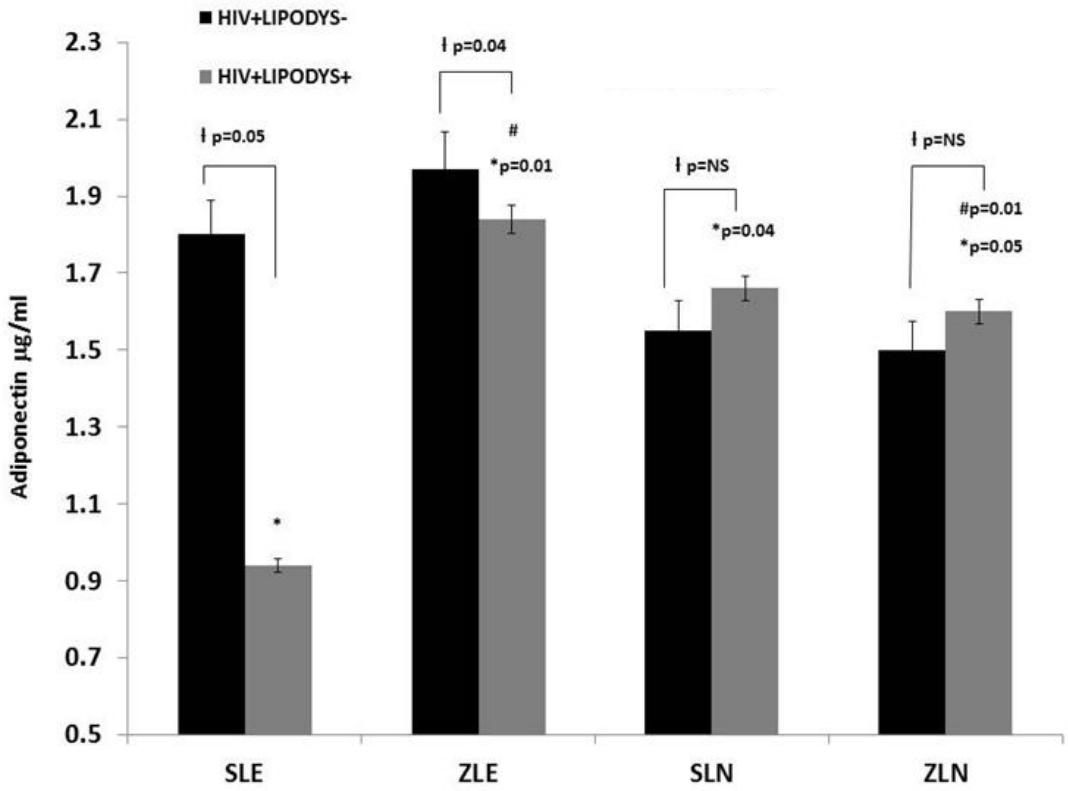

Figure 1. Levels of Adiponectin in patients with and without lipodystrophy in the antiretroviral treatment regimens groups. Statistical comparisons made between patients in the 4 different ART regimens among patients with and without lipodystrophy. Data shown is mean ± standard deviation values in each group. A p value of <0.05 is considered statistically significant.
No significance between the 4 ART in HIV+LIPODYS- patients. Significant differences in the HIV+LIPODYS+ patients when *comparisons made between SLE vs. ZLE, SLN and ZLN.
#Comparisons between ZLE vs. ZLN.
ǂComparisons between HIV+LIPODYS- vs. HIV+LIPODYS+ patients within each ART group.

some patients had clinical features of lipodystrophy (lipoatrophy and/or lipohypertrophy), they did not have overt dyslipidemia probably reflecting partial lipodystrophy.

HIV-1-infected patients with lipodystrophy usually developed insulin resistance (IR) and dyslipidemia resulting in a metabolic syndrome. Although, we did not observe any significant differences in blood glucose levels in patients with and without lipodystrophy, we observed increased insulin levels and increased insulin resistance in the patients with lipodystrophy in all the four ART groups (Table 3) indicating a tendency to-wards development of the metabolic syndrome in patients with lipodystrophy irrespective of the ART type. Metabolic syndrome is a condition wherein there is an aggregation of fat redistribution and glucose and lipid metabolism alterations that confers an increased risk of cardio-vascular disease (Sevastianova et al., 2008; Brown and Confrancesco, 2006; Stankov and Behrens, 2010; Gougeon et al., 2004; Tanwani and Mokshagundam, 2003; Lagathu et al., 2005). The etiology of IR among treated HIV-infected patients is most likely multifactorial and includes primarily a genetic influence, side effects of certain antiretroviral drugs and alterations in body fat distribution (Sevastianova et al., 2008; Brown and Confrancesco, 2006; Stankov and Behrens, 2010; Gougeon et al., 2004; Tanwani and Mokshagundam, 2003; Lagathu et al., 2005). The expression of the cytokine TNF-α may be involved in adipose tissue pathology associated with lipodystrophy. The presence or absence of the TNF-α 238G/A SNP may modulate TNF-α production modulating inflammatory response associated with the metabolic syndrome. We therefore evaluated the levels of TNF-α and adiponectin which is a novel adipocyte-specific protein, which plays a role in the development of insulin resistance and atherosclerosis.

It is believed that the expression of TNF-α may be involved in adipose tissue pathology associated with

lipodystrophy (Sankale et al., 2006; Bedimo, 2008; Caron-Debarle et al., 2010b). We have recently shown that the presence of the TNF-α 238G/A SNP increases the risk of lipodystrophy progression as indicated by development of lipoatrophy/dyslipidemia and insulin resistance in the HIV-1 Clade C infected patient cohort from Western India (manuscript in review). We observed a significant increase in the expression of TNF-α 238G/A SNP in patients with lipodystrophy as compared to patients without lipodystrophy in all the 4 ART regimen groups (Table 4). TNF-α is thought to be the key cytokine involved in the pathogenic and metabolic events associated with HIV-1 infection and the TNF-α SNP could be used as clinical prognostic markers to predict the development of HIV-1 associated lipodystrophy. TNF-α expression may modulate the downstream effects of antiretroviral therapy in adipose tissue, where this cytokine is known to play an important role in determining insulin sensitivity and in adipocyte differentiation. ART therapy along with the increased levels of the inflammatory cytokines such as TNF-α is associated with a decrease in adiponectin (Bedimo, 2008; Caron-Debarle et al., 2010b). Altered adiponectin secretion could result from patients' exposure to NNRTI and NRTIs and leads to altered adipocyte differentiation and insulin resistance. These alterations are probably involved in the metabolic changes that we have observed in our HIV+ LIPODYS+ patient cohort.

We evaluated the effects of the various ART on adiponectin levels in patients with and without lipodystrophy and found the lowest levels of adiponectin in patients with lipodystrophy in the SLE group. Adiponectin levels were lower in the ZLE group as well but were marginally higher in the SLN and ZLN treatment groups respectively (Figure 1) suggesting that these different ARV regimens have different effects on the adiponectin secretion. Low levels of adiponectin reflect fat atrophy which may contribute to insulin resistance and its metabolic complications, while higher levels of adiponectin may be a reflection of partial lipodystrophy.

Further, our observation of decreased adiponectin levels in patients with lipodystrophy who were on the SLE regimen, is in agreement with earlier reports that stavudine and efavirenz are both likely to increase the risk of lipodystrophy by causing dyslipidemia. The result of our correlation analysis between ART regimen groups also shows that ART significantly modulates that adiponect in levels. NRTIs are able to induce mitochondrial dysfunction and to modify adipocyte phenotype and adipose tissue pattern of secretion of cytokines as TNF-α and adiponectin, probably through the production of reactive oxygen species. These ARTs may also act on adipocytes, by altering its differentiation and insulin sensitivity and also the pattern of secretion of adiponectin by adipose tissue (Bedimo, 2008; Caron-Debarle et al., 2010b; Giralt et al., 2006; Kosmiski et al., 2003; Addy et al., 2003). Adiponectin also has direct effects on hepatic lipid metabolism via its truncated COOH-terminal globular domain which increases fatty acid oxidation and energy expenditure, these increases of muscular fatty acid combustion will consequently improve dyslipidemia byenhancing lipid clearance from the circulation (Grunfeld et al., 1992; Villarreal-Molina and Anutuna-Puente, 2012). Studies have shown that in addition to its effect on lipid metabolism, Adiponectin also has direct anti-atherogenic effects (Grunfeld et al., 1992; Villarreal-Molina and Anutuna-Puente, 2012; Kanhai et al., 2013; Ohashi et al., 2012).

Conclusion

In conclusion, the mechanisms that underlie ART mediated dyslipidemia remain incompletely understood, and different ARTs may induce dyslipidemia through various distinct mechanisms. The pathogenesis of HIV-1 associated lipodystrophy is multifactorial. Additionally, it is difficult to accurately access the individual contribution of each antiretroviral drug to lipodystrophy in majority of patients who are on combination therapy such as ART. These are the major limitation of this study. Data from our study suggest that Adiponectin replacement therapy may be an effective strategy in the treatment of dyslipidemia in patients who are on a prolonged ART regimen. Further, specific TNF-α polymorphisms are associated with the development of lipodystrophy in HIV/AIDS patients. The link between the TNFα 238G/A promoter SNP and development of lipodystrophy is probable, however studies showing reproducibly high predictive values of this SNPs association with clinically relevant and well defined metabolic outcome are warranted. Effect of genetic determinants on dyslipidemia may provide insight into potential mechanisms that underlie HIV lipodystrophy progression and will be useful in treating individual patients as clinical medicine advances steadily towards a personalized medicine approach.

Abbreviations: ART, Antiretroviral therapy; **SNP,** single nucleotide polymorphism; **PI's,** protease inhibitors; **NRTI,** nucleoside reverse transcriptase inhibitor; **NNRTI,** non-nucleoside reverse transcriptase inhibitors**; EFV,** efavirenz; **ZDV,** zidovudine; **NVP,** nevirapine; **HOMA,** homeostatic model assessment; **IR,** insulin resistance.

Conflicts of interest

All authors report no conflict of interest.

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