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Increased trunk fat along with decreased peripheral fat as an important predictor of hypertriglyceridaemia & hypercholesterolaemia in Indians with HIV infection

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***Background & objectives*: Dyslipidaemia is a major contributor to cardiovascular morbidity, which is increased in HIV. Data on dyslipidaemia in Indians with HIV are scant. This study was undertaken to determine the predictors of dyslipidaemia and lipoatrophy in Indians with HIV infection and their relation with body composition parameters.**

***Methods*: A total of 382 consecutive patients with HIV infection were screened, of whom 257 clinically stable patients, without any acute comorbidity, having at least one year follow up underwent biochemical and DEXA analysis.**

***Results*: The most common dyslipidaemia was hypertriglyceridaemia (47.08%), followed by hypercholesterolaemia [total cholesterol (TC)] (38.91%) and low high-density lipoprotein (HDL) cholesterol (38.52%), in patients having median age 37 (32-42) yr and HIV duration 57 (33-101) months. Patients with at least one dyslipidaemia (78.99%) had significantly higher insulin resistance (IR), per cent body fat, per cent trunk fat (PTF) and trunk limb fat ratio (TLFR). Baseline CD4 count and delta CD4 count (change in CD4 count 6-12 months following ART) had significant inverse correlation with triglycerides and TC. Patients with highest triglycerides and cholesterol quartiles had significantly higher immune reconstitution, metabolic syndrome, IR, trunk fat mass (FM), PTF and TLFR, with comparable total FM. Logistic regression revealed that body mass index, HIV duration and PTF were independent predictors of hypertriglyceridaemia, with only PTF being significant predictor of hypercholesterolaemia. Every unit increase in PTF was associated with 13 and 4.1 per cent increased hypertriglyceridaemia and hypercholesterolaemia. Lipoatrophy was present in 8.57 per cent patients and was a poor predictor of dyslipidaemia.**

***Interpretation & conclusions*: High occurrence of dyslipidaemia was observed in patients with HIV on anti retroviral therapy. Central adiposity (TFM) was the most important predictor of dyslipidaemia in these patients.**

Key words Bone mass - fat mass - HIV - hypercholesterolaemia - hypertriglyceridaemia - lean mass

HIV patients are known to be associated with increased cardiovascular morbidity and mortality[1]. Dyslipidaemia [increased triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) along with decreased high-density lipoprotein cholesterol (HDL-C)] is known to occur in HIV. Anti-retroviral therapy (ART) has been associated with dyslipidaemia and HIV lipodystrophy in different ethnic groups[1]. HIV lipodystrophy is a spectrum disorder consisting of lipoatrophy, lipohypertrophy and mixed patterns, characterized by peripheral fat atrophy with central fat accumulation. Data on lipoatrophy and its relation with dyslipidaemia in Indians with HIV infection are scant. In a study of 168 patients on ART from Chennai, India, increased levels of TC, LDL-C and low HDL-C were documented in 26, 23 and 23 per cent patients, respectively[2]. The occurrence of lipodystrophy among Indians has been reported to range from 23 to 61 per cent[3,4]. Heterogeneity in definitions used for the assessment may explain this variance.

The relationship between body composition abnormalities and dyslipidaemia among Indians with HIV infection is not well known. Hence, the aim of this study was to determine the burden and predictors of dyslipidaemia and lipoatrophy in asymptomatic patients with HIV infection with good immune function on ART. This study was also aimed to evaluate the relation of dyslipidaemia and lipoatrophy with alterations in body composition [fat mass (FM), lean mass (LM) and bone mass] in these patients.

Material & Methods

Consecutive ambulatory patients, with serologically documented HIV infection, in stable clinical condition, without any acute, severe illness, attending the ART clinic of Post-Graduate Institute of Medical Education and Research (PGIMER) and Dr. Ram Manohar Lohia (RML) Hospital, New Delhi, during August 2015 to November 2016 were considered. Severely ill patients with multiple comorbid states, which would warrant hospital admission, patients with known endocrinopathies (hypogonadism, hypopituitarism, hypothyroidism and hypocortisolism) were excluded. Patients with a history of hospital admissions in the last two months were also excluded. Patient records were reviewed, and those having clinical data of at least one year follow up were further evaluated. Patients with available CD4 cell counts at diagnosis and at first follow up (6 months after diagnosis) were included. The study protocol was explained to the patients and only those who gave informed written consent were included. The institutional ethics committee of PGIMER and Dr. RML Hospital approved the study protocol.

Data were collected regarding duration of HIV infection and details of ART. Patients underwent detailed clinical assessment, including anthropometry. Lipoatrophy was assessed by consensus between two doctors using a standardized visual grading scale as described by Carr *et al*[5,6]. The following grades were defined: Grade 0 - No fat changes; Grade 1 - Possible minor changes, noticeable only on close inspection; Grade 2 - Moderate changes, unequivocally noticeable only to an experienced clinician or a close relative who knows the patient well; Grade 3 - Major changes, readily noticeable to an uninformed observer. Visually obvious lipoatrophy (presence of significant lipoatrophy) was defined as a score of 2 or more[5,6]. Face, arms, legs and buttocks were assessed for loss of subcutaneous fat, resulting in a lean, muscular appearance of limbs and face, abnormally prominent limb veins and loss of gluteal fat pad with reduction in buttock size and loss of gluteal contour. In case of discrepancy in the scores of the two doctors, the lower score was used for analysis to increase the specificity of having lipoatrophy. Patients were called the subsequent day in fasting state for blood sampling. Blood samples (5 ml) were collected in plain and ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson, USA). Serum was separated from blood collected in a plain vacutainer and processed immediately for routine biochemical analysis, and one aliquot of serum was stored at −20°C.

Chemiluminescent microparticle immunoassay (VITROS® ECiQ Immunodiagnostic System, Johnson & Johnson, USA) was used for estimation of 25-hydroxyvitamin-D (25OHD) and fasting insulin. Serum 25OHD assay had analytical sensitivity of 19.97 nmol/l, analytical range of 19.97-374.40 nmol/l, intra- and inter-assay coefficient of variation (CV) of 3.4 and 5.5 per cent, respectively. Serum insulin assay had analytical sensitivity: 2 μIU/ml, analytical range of 2-350 μIU/ml; intra- and inter-assay CV of 5.1 and 7.6 per cent, respectively. Serum lipid profile and fasting glucose were tested using clinical chemistry autoanalyzer based on dry chemistry micro-slide technology (VITROS® 350 chemistry system, Johnson & Johnson, USA). CD4 cell count was performed using flow cytometry (Becton Dickinson Immunocytochemistry Systems, USA).

Dual-energy X-ray absorptiometry (DEXA; Discovery Wi Series, Serial Number: 84571; Hologic Inc., Waltham, MA, USA) was used to measure whole-body bone mineral content (BMC) (kg), total body fat (kg), percentage FM (%), FM (kg), LM (kg), trunk FM (kg), limb FM (kg), limb LM (kg), android fat (kg) and gynoid fat (kg). Body composition analyses of soft tissues were performed using the QDR2000 product software, version 7.10A (Hologic, Boston, MA, USA). Quality control procedures were done as per the manufacturer's recommendations. The instrument was calibrated on a daily basis, using phantom provided by manufacturer. Reproducibility of DEXA measurements was derived from the root mean square standard deviation of two repeat measurements[7,8]. Technique precision for body composition variables was 12.32 g for BMC (0.96% CV), 166.3 g for LM (0.74% CV) and 156.2 g for FM (0.72% CV). The manufacturer's appointed service engineer reviewed the calibration data and did scanner maintenance check to ensure system's performance before at the beginning and at the end of the study.

Immune reconstitution inflammatory syndrome (IRIS) in HIV-infected patients is characterized by clinical deterioration secondary to re-establishment of immunity following ART[1]. It is usually observed in patients with low baseline CD4 count, which increases rapidly following ART initiation. ART has been linked to increased systemic inflammation and autoimmunity[1-3]. IRIS has been defined as CD4 count >200 cells/µl in patients who previously had CD4 counts <100-200 cells/µl with associated clinical phenotype and systemic inflammation[3,4]. Hence, patients in our study with baseline CD4 counts <200 cells/µl, which increased to >200 cells/µl at first follow up following ART initiation (6 months) were defined to have immune reconstitution. Raised serum TC, triglycerides, LDL-C were defined as >200 mg/dl, >150 mg/dl and >130 mg/dl, respectively. Low HDL-C was defined as <40 mg/dl in males and <50 mg/dl in females[8].

In a previous study from India, dyslipidaemia was documented in 26 per cent of HIV patients[2]. Hence, for keeping a power of 80 per cent and type-I error at 5 per cent, the sample size was calculated to be 151 patients for accurate assessment of dyslipidaemia.

Statistical analysis: Normality of the distribution of variables was checked using the Kolmogorov-Smirnov test. Normally distributed variables were expressed as mean±standard deviation. All non-normally distributed variables were expressed as median (25-75th percentile). Independent *t* test and Wilcoxon rank sum test were done for normally distributed and skewed variables, respectively. Chi-square test was used for categorical variables. Binary logistic regression was initially performed with all parameters, which are likely to influence occurrence of hypertriglyceridaemia and hypercholesterolaemia. Parameters with $P<0.2$ were included in the final model. Statistical Package for the Social Sciences (SPSS) version 20 (Chicago, IL, USA) was used for data analysis.

Results

In this cross-sectional study, a total of 382 consecutive patients with HIV infection were screened, of whom 257 patients (male:female=181:76), who fulfilled all inclusion and exclusion criteria, and gave informed written consent underwent biochemical and DEXA analysis. Sixty five patients were excluded as they were receiving treatment for tuberculosis, 19 patients were on antiviral therapy for hepatitis B, 11 were on antifungal therapy and three patients were on antiviral therapy for hepatitis C. Twenty seven patients had a history of acute febrile illness within the last one month, necessitating use of antibiotics, or had a history of hospital admission within the last one month, hence excluded.

The median age and duration of HIV infection (duration since they were detected to have seropositive HIV infection) among the patients was 37 (32-42) years and 57 (33-101) months, respectively. Eighty patients (31.12%) had history of tuberculosis and 69 patients (26.84%) had history of immune re-constitution. Nine and two patients had hepatitis B and hepatitis C co-infection, respectively. As per the National AIDS Control Organization (NACO) guidelines[2,3], zidovudine, lamivudine and/or tenofovir were the nucleoside reverse transcriptase inhibitors (NRTIs) received by the patients; nevirapine or efavirenz were non-NRTIs; atazanavir or ritonavir was the protease inhibitors (PI) received by the patients.

Dyslipidaemia was common in patients with HIV infection with 203 of the 257 patients (78.99%) evaluated in this study having at least one lipid abnormality. The most common lipid abnormality observed was hypertriglyceridaemia (n=121; 47.08%), followed by raised cholesterol (hypercholesterolaemia) (n=100; 38.91%), raised HDL-C (n=99; 38.52%) and

raised LDL-C (n=74; 28.79%). Clinically, significant lipoatrophy was present in 22 patients (8.57%). Hypertension was documented in 19 patients (7.39%) and metabolic syndrome (MetS) (as per NCEP-ATP-III criteria[5,6]) was diagnosed in 73 patients (28.40%).

As compared to those with a normal lipid profile, patients with at least one lipid abnormality had significantly higher insulin resistance (IR) (higher HOMA-IR and lower QUICKI) and significantly lower estimated β-cell function (HOMA-β), (Table I).

Table I. Clinical, anthropometric, metabolic and body composition profile of patients with HIV infection with at least one lipid abnormality as compared to those with a normal lipid profile

Parameter	At least one lipid abnormality (n=203)	Normal lipid profile (n=54)	*P*
Age (yr)	37.37±7.29	36.94±8.19	0.708
Sex (male:female)	135:68	46:8	0.008
BMI (kg/m^2)	22.66±4.2	21.83±3.63	0.200
History of tuberculosis	61	19	0.201
History of immune reconstitution	53	16	0.318
NRTI use	161	40	0.414
NNRTI use	154	40	0.381
Protease inhibitors use	6	1	0.731
Total cholesterol (mg/dl)[a]	198 (165-216)	160 (140-178)	<0.001
Triglycerides (mg/dl)[a]	159 (132-211)	119 (79-132)	<0.001
LDL-C (mg/dl)[a]	118 (86-142)	94 (79-111)	<0.001
HDL-C (mg/dl)[a]	44 (35-52)	54 (45-59)	<0.001
FBG (mg/dl)[a]	91 (85-99)	88 (74-92)	0.003
HOMA-IR[a]	1.86 (1.46-2.83)	1.76 (1.32-2.09)	0.018
QUICKI	0.34 (0.32-0.36)	0.35 (0.34-0.36)	0.020
HOMA-β[a]	120.66 (116-126.96)	123.96 (115.1-127.6)	0.045
Duration of HIV infection (months)[a]	56.5 (34.25-95.75)	66.5 (24.75-107.25)	0.746
Baseline CD4 count (pre ART) (cell/µl)[a]	191 (113-366)	162 (112-276)	0.095
CD4 count (6 months post ART) (cell/µl)[a]	315 (199-412)	274 (210-423)	0.258
Current CD4 count (cell/µl)	393 (265-548)	393 (317-494)	0.614
Delta CD4 count (cells/µl)	79 (0-199)	86 (5-252)	0.413
25OHD (ng/ml)[a]	18.4 (12.5-21.95)	17.6 (14.1-21.02)	0.085
Total fat mass (kg)[a]	15.89 (12.02-22.71)	14.91 (12.07-18.55)	0.071
Total fat per cent	27.7 (22.4-37.0)	26.10 (22.57-30.05)	0.036
Total lean mass (kg)[a]	39.57 (32.12-47.53)	41.38 (37.99-46.46)	0.811
Total bone mineral content (kg)[a]	2.05 (1.77-2.26)	2.17 (1.90-2.45)	0.890
Trunk fat mass (kg)[a]	8.72 (7.26-11.18)	6.80 (5.17-8.24)	0.002
Limb fat mass (kg)[a]	7.05 5.05-9.02)	6.27 (5.11-8.36)	0.574
TLFR[a]	1.27 (1.01-1.54)	0.94 (0.78-1.06)	0.004
PTF (%)[a]	53.11 (46.92-59.32)	44.83 (40.47-48.05)	0.117
Android/gynoid (A/G) ratio[a]	0.60 (0.49-0.84)	0.68 (0.45-0.94)	0.976

[a]All non-normally distributed variable expressed as median (25-75th percentile); LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; ART, anti-retroviral therapy; HOMA, homoeostatic model of insulin resistance; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; QUICKI, quantitative insulin sensitivity check index; MetS, metabolic syndrome; 25OHD, 25-hydroxyvitamin-D; Normality of variable distribution checked using Kolmogorov-Smirnov test; normally distributed variables expressed as mean±SD; SD, standard deviation; BMI, body mass index; PTF, per cent trunk fat; TLFR, trunk limb fat ratio

Among body composition parameters, patients with at least one lipid abnormality had significantly higher per cent body fat, per cent trunk fat (PTF) and trunk limb fat ratio (TLFR) (Table I). Baseline CD4 count had inverse correlation with serum triglycerides (σ=−140; *P*=0.039), TC (σ=−129; *P*=0.046) and HDL-C (σ=−0.122; *P*=0.074). Delta CD4 count (change in CD4 count 6-12 months following ART initiation, as compared to baseline) had a positive correlation with serum triglycerides (σ=0.112; *P*=0.083) and TC (σ=0.123; *P*=0.042) and HDL-C (σ=0.094; *P*=0.101).

Analysis of the clinical, metabolic (IR) and body composition parameters as per the quartiles of serum triglycerides revealed that patients in the highest quartile of triglycerides had significantly higher history of immune reconstitution, higher BMI, MetS, fasting blood glucose and IR (HOMA-IR and QUICKI) (Table II). Patients with hypertriglyceridaemia had the greater increase in CD4 count following initiation of ART (delta CD4 count), *viz*. a better initial response to ART (Table II). In spite of having comparable total FM, patients in the highest quartiles of serum triglycerides had a significantly higher trunk FM, PTF, higher TLFR and higher android/gynoid (A/G) ratio. Binary logistic regression analysis revealed that BMI, duration of HIV infection and PTF were the independent predictors of hypertriglyceridaemia in patients with HIV infection (Table III). Increased disease duration was associated with decreased risk, whereas increased BMI and PTF were associated with increased risk of hypertriglyceridaemia. Every unit increase in PTF was associated with 13 per cent increased risk of hypertriglyceridaemia (Table III).

Analysis of the clinical, metabolic (IR) and body composition parameters as per the quartiles of serum TC revealed that patients in the highest quartile of TC had significantly higher occurrence of hypertension, MetS, fasting blood glucose, IR (HOMA-IR and QUICKI) and significantly lower estimated β-cell function (HOMA-β) (Table IV). Patients with increased TC had the greater increase in CD4 count following initiation of ART (delta CD4 count), *viz*. lower baseline CD4 count with a better initial response to ART (Table IV). In spite of having comparable total FM and total body fat per cent, patients in the highest quartiles of serum TC had a significantly higher trunk FM and higher TLFR (Table IV). Binary logistic regression analysis revealed that PTF was the only significant independent predictor of occurrence of hypercholesterolaemia in patients with HIV infection (Table V). Every unit increase in PTF was associated with 4.2 per cent increased risk of hypercholesterolaemia (Table V).

Patients with lipoatrophy had significantly higher occurrence of MetS. TC, LDL-C, fasting glucose, HOMA-IR and A/G were significantly higher in patients with lipodystrophy as compared to those without (Table VI). Body composition parameters such as FM, LM and bone mass were comparable in HIV patients with lipoatrophy as compared to those without lipoatrophy.

Discussion

In our study dyslipidaemia was observed in 79 per cent of patients, with hypertriglyceridaemia being the most common type (47.08%), followed by hypercholesterolaemia (38.91%). These rates were much higher than that reported in the general Indian population[2-4]. In a cohort of 16,607 individuals recruited from different parts of India, the Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study showed that the prevalence of hypertriglyceridaemia and hypercholesterolaemia was 29.5 and 13.9 per cent, respectively[9]. Impact of ART on lipid profile can be highlighted from the observation that the occurrence of hypercholesterolaemia (TC >200 mg/dl) and increased LDL-C (LDL-C >130 mg/dl) increased from 1 to 26 per cent and 3 to 23 per cent, respectively, before and after ART in a group of 168 patients[2]. Hypertriglyceridaemia, hypercholesterolaemia, low HDL-C and MetS have been identified as important therapeutic targets by the National Cholesterol Education Program (NCEP) with regards to cardiovascular morbidity reduction[8,10]. The NCEP recommends initially targeting therapy to lower triglycerides, in patients with severe hypertriglyceridaemia before turning to LDL-C to further lowering of cardiovascular risk[10]. An important observation of this study was the high occurrence of low HDL-C observed in 38.55 per cent patients. A similar high occurrence of low HDL-C (43%) has been reported from African countries such as South African, Nigeria and Uganda, which is in contrast to reports from high-income countries (America and Europe)[11]. Low HDL and hypertriglyceridaemia have been observed to be promising short-term mortality markers in inpatients

Table II. Clinical, anthropometric, metabolic and body composition profile of patients with HIV infection as per the quartiles of serum triglycerides

Parameter	Serum triglycerides				*P*
	Quartile-1 69-119 mg/dl (n=72)	Quartile-2 119-145 mg/dl (n=59)	Quartile-3 145-196 mg/dl (n=64)	Quartile-4 196-660 mg/dl (n=62)	
Age (yr)	35.61±6.79	37.67±7.75	38.57±6.81	37.51±8.31	0.124
Sex (male:female)	47:25	40:19	44:20	50:12	0.230
BMI (kg/m^2)	21.58±3.43	21.17±4.10	23.0±4.41	24.27±3.82	<0.001
History of tuberculosis (%)	23 (31.94)	13 (22.03)	25 (39.06)	19 (30.64)	0.267
History of immune reconstitution (%)	15 (20.83)	9 (15.25)	17 (26.56)	28 (45.16)	<0.001
Hypertension (%)	2 (2.77)	6 (10.15)	5 (7.81)	6 (9.67)	0.402
MetS (%)	5 (6.94)	13 (22.03)	30 (46.80)	25 (40.32)	<0.001
Lipoatrophy	5	5	8	4	0.602
NRTI use (%)	53	48	55	45	0.656
NNRTI use (%)	51	47	53	43	0.644
Protease inhibitors use (%)	4	1	2	0	0.219
Total cholesterol (mg/dl)[a]	157 (137-183)	198 (174-210)	199 (150-230)	206 (182-243)	<0.001
LDL-C (mg/dl)[a]	95 (80-121)	127 (104-140)	120 (78-152)	108 (78-144)	0.015
HDL-C (mg/dl)[a]	40 (34-52)	46 (38-52)	48 (43-56)	47 (41-56)	<0.001
Elevated total cholesterol (%)	8 (11.11)	26 (44.06)	32 (50)	34 (54.83)	<0.001
Elevated LDL-C (%)	12 (16.67)	17 (28.81)	26 (40.62)	19 (30.64)	0.022
Low-HDL-C (%)	38 (52.78)	25 (42.37)	20 (31.25)	16 (25.80)	0.010
FBG (mg/dl)[a]	87 (81-89)	87 (82-92)	91 (87-108)	89 (85-102)	<0.001
HOMA-IR[a]	1.68 (1.28-1.95)	1.71 (1.22-2.36)	2.22 (1.71-3.98)	2.02 (1.46-3.52)	<0.001
QUICKI	0.35 (0.34-0.36)	0.35 (0.33-0.37)	0.34 (0.31-0.35)	0.34 (0.31-0.36)	<0.001
HOMA-β[a]	117.1 (114-124.3)	121.3 (116.9-128.9)	121.6 (118.2-126.7)	125.3 (115.6-129.3)	0.585
Duration of HIV infection (months)[a]	78 (43-118)	48 (26-81)	54 (31-95)	53 (35-101)	0.019
Baseline CD4 count (pre ART) (cell/µl)[a]	248 (145-354)	184 (111-368)	187 (113-369)	157 (106-219)	0.221
CD4 count (6 months post ART initiation) (cell/µl)[a]	306 (231-432)	256 (177-399)	286 (182-439)	329 (246-384)	0.285
Current CD4 count (cell/µl)	393 (264-611)	424 (245-677)	402 (287-510)	393 (257-482)	0.866
Delta CD4 count (cells/mm^3)	92 (0-172)	49 (−28-141)	79 (−15-181)	199 (44-230)	0.096
25OHD (ng/ml)[a]	18.53 (12.7-21.5)	17.65 (13.5-21.8)	17.72 (12.50-22.27)	18.4 (12.9-24.6)	0.442
Total fat mass (kg)[a]	15.36 (13.29-18.80)	17.19 (12.02-20.66)	14.93 (9.26-19.72)	16.23 (13.64-24.65)	0.561
Total fat per cent (%)	27.6 (23.47-38.0)	27.95 (23.9-38.15)	29.5 (18.6-36.5)	27.05 (22.4-30.90)	0.187
Total lean mass (kg)[a]	37.80 (29.89-44.20)	38.05 (30.26-46.11)	39.40 (28.88-47.07)	45.18 39.01-50.44)	<0.001
Total bone mineral content (kg)[a]	2.06 (1.79-2.35)	2.05 (1.77-2.20)	1.97 (1.83-2.19)	2.11 (1.82-2.28)	0.089
Trunk fat mass (kg)[a]	7.26 (4.92-8.22)	8.20 (6.53-9.97)	9.43 (3.73-12.06)	9.15 (7.84-12.06)	0.001
Limb fat mass (kg)[a]	7.28 (5.31-9.66)	8.01 (6.19-8.88)	8.36 (2.63-10.17)	6.11 (4.72-8.77)	0.655
TLFR[a]	0.80 (0.68-1.02)	1.02 (0.99-1.09)	1.14 (0.90-1.22)	1.52 (1.36-1.82)	<0.001
PTF (%)[a]	44.29 (38.13-53.63)	46.85 (45.86-50.56)	50.55 (38.22-51.97)	56.84 (53.11-61.61)	<0.001
Android/gynoid (A/G) ratio[a]	0.54 (0.42-0.67)	0.74 (0.46-0.81)	0.57 (0.51-0.62)	0.85 (0.55-1.04)	<0.001

[a]All non-normally distributed variable expressed as median (25-75th percentile). Abbreviations are as given in Table I

Table III. Binary logistic regression analysis showing factors that independently predict the occurrence of hypertriglyceridaemia in patients with HIV infection

Variable	β	Exp(β)	*P*
Age	−0.022	0.978	0.818
BMI	1.041	2.832	0.039
Duration of HIV infection	−0.073	0.929	0.018
Immune reconstitution	−3.484	0.031	0.151
Delta CD4 count	−0.026	0.975	0.060
Total lean mass	0.000	1.000	0.973
PTF	0.124	1.132	0.044
Android/gynoid (A/G) ratio	−5.351	0.005	0.298

Binary logistic regression was initially performed with all parameters which are likely to influence the occurrence of hypertriglyceridaemia (age, sex, BMI, duration of HIV infection, baseline CD4 count, delta CD4 count, history of tuberculosis, immune reconstitution, individual antiretroviral agents received by the patient, total lean mass, total fat mass, PBF, PTF, truck limb fat ratio, android/gynoid ratio). Parameters with $P<0.2$ were included into the final model; Exp(β): exponentiation of the β coefficient, change in odds ratio with 1 unit change in predictor variable; for categorical variable immune reconstitution, patients with immune reconstitution were taken as the reference group. ART: antiretroviral therapy; PTF, per cent trunk fat; PBF, per cent body fat; PTF, per cent trunk fat; BMI, body mass index

with HIV infection[12]. A study from New Delhi, India, documented 38.3 per cent of children with HIV on ART to have dyslipidemia[13].

In spite of having a comparable total FM, patients with at least one lipid abnormality, hypertriglyceridaemia or hypercholesterolaemia consistently had a higher PTF and TLFR. This highlights that it is the relative increase in visceral adiposity, with a relative paucity of peripheral adiposity that contributes to the genesis of lipid abnormalities in patients with HIV infection. This hypothesis was further supported by the observation that binary logistic regression consistently demonstrated PTF to be the most important independent predictor for both hypertriglyceridaemia and hypercholesterolaemia. A study in African children on ART has similarly demonstrated increased trunk fat with loss of peripheral subcutaneous fat to be associated with hypertriglyceridaemia[14].

This increased central adiposity and hypercholesterolaemia were associated with increased fasting glucose, IR and decreased beta-cell function, highlighting the adverse impact of abnormal body fat distribution on long-term glycaemic outcomes. Increased visceral adiposity has been linked to increased systemic inflammation [tumour necrosis factor α (TNF α), interleukins (ILs)], decreased glucose transporter expression, decreased activity of gamma receptors activated by peroxisome proliferator that lead to dyslipidaemia, IR, hypertension and atherosclerosis[15]. TNFα 238G/A SNP occurrence has been documented to be two-fold higher in patients with HIV lipodystrophy as compared to those without lipodystrophy[16]. TNF-α 238G/A SNP has previously been linked with increased circulating TNFα levels, increased insulin resistance, dyslipidaemia, and adverse glycaemic outcomes in prediabetes[17].

Our study showed that HIV patients having a poor immune function at the time of diagnosis of HIV infection, along with a better initial response to ART (*viz*. low baseline CD4 count with greater improvement in CD4 count following ART initiation), had a higher risk of developing one or more lipid abnormalities later in life. This was supported by the observation of inverse correlation of current lipid parameters with baseline CD4 count and positive correlation with delta CD4 count. A more rapid improvement in immune function following ART initiation may be linked to increased abnormal fat distribution (increased truncal adiposity) later in life, which may contribute to the development of dyslipidaemia. This was in accordance with the observation that patients in the highest quartile of serum triglycerides had significantly higher history of immune reconstitution following ART initiation.

All these observations highlight the poor basal immune function and role of ART in the development of abnormal fat distribution in HIV patients (increased central adiposity with peripheral fat loss). Among the ART medications, PIs and stavudine have been most commonly linked to dyslipidaemia[1,18]. On regression analysis, none of the specific ART drugs received by the patients was associated with dyslipidaemia. It must be highlighted that none of the patients in our study were on stavudine, and use of PIs was negligible (2.7%). Arpadi *et al*[19] and a study by the European Pediatric Group of Lipodystrophy[20] also documented significant associations between poorer virological and immunological status at baseline with the occurrence of dyslipidaemia and abnormal body fat distribution later in life. Flint *et al*[21] have

Table IV. Clinical, anthropometric, metabolic and body composition profile of patients with HIV infection as per the quartiles of serum total cholesterol

Parameter	Serum total cholesterol				*P*
	Quartile-1 90-154 mg/dl (n=68)	Quartile-2 154-188 mg/dl (n=59)	Quartile-3 188-212 mg/dl (n=66)	Quartile-4 212-313 mg/dl (n=64)	
Age (yr)	35.34±7.29	35.52±7.25	37.43±6.93	40.85±7.19	<0.001
Sex (male:female)	49:19	38:21	45:21	49:15	0.506
BMI (kg/m^2)	21.83±4.09	22.32±3.8	22.31±3.58	23.52±4.69	0.123
History of tuberculosis	16	17	22	25	0.250
History of immune reconstitution	16	12	17	24	0.132
Hypertension	1	2	10	6	0.006
MetS	3	4	20	46	<0.001
Lipoatrophy	4	3	6	9	0.236
Triglycerides (mg/dl)[a]	119 (79-165)	135 (105-173)	143 (123-196)	176 (151-287)	<0.001
LDL-C (mg/dl)[a]	75 (61-87)	103 (94-111)	127 (118-131)	151 (141-165)	<0.001
HDL-C (mg/dl)[a]	48 (37-59)	45 (38-54)	44 (38-52)	45 (40-53)	0.675
Elevated triglycerides	25	20	28	48	<0.001
Elevated LDL-C	0	0	18	56	<0.001
Low-HDL-C	22	23	32	22	0.217
FBG (mg/dl)[a]	86 (79-93)	88 (81-95)	88 (86-94)	89 (85-108)	0.004
HOMA-IR[a]	1.63 (1.27-2.11)	1.76 (1.46-2.52)	1.83 (1.48-2.62)	2.01 (1.45-3.82)	0.005
QUICKI	0.35 (0.34-0.36)	0.35 (0.33-0.36)	0.34 (0.32-0.36)	0.34 (0.31-0.36)	0.010
HOMA-β[a]	123.2 (116.05-128.58)	118.32 (114.5-125.6)	121 (115-126)	120 (117-128)	0.025
Duration of HIV infection (months)[a]	55 (38-107)	79 (44-103)	56 (32-85)	52 (23-110)	0.224
Baseline CD4 count (pre ART) (cell/µl)[a]	203 (117-359)	177 (140-368)	169 (106-350)	168 (110-332)	0.189
CD4 count (6 months post ART initiation) (cell/µl)[a]	315 (216-420)	250 (160-397)	357 (199-412)	291 (231-483)	0.971
Current CD4 count (cell/µl)	391 (291-480)	439 (266-527)	367 (191-522)	436 (308-604)	0.127
Delta CD4 count	71 (0-203)	0 (−41-118)	94 (41-181)	123 (26-230)	0.047
25OHD (ng/ml)[a]	16.5 (11.2-21.1)	18 (15-21.60)	17.06 (12.1-21.6)	21.1 (17.87-24.1)	<0.001
Total fat mass (kg)[a]	14.93 (11.73-19.26)	16.81 (13.44-22.70)	14.49 (12.10-21.74)	15.23 (12.89-22.68)	0.751
Total fat per cent	25.1 (22.2-33.6)	30.9 (23.2-36.5)	28.25 (24.80-38.05)	27.6 (19.8-34.05)	0.440
Total lean mass (kg)[a]	39.42 (32.22-47.66)	42.85 (30.99-48.67)	38.81 (32.83-44.14)	40.14 (37.17-50.93)	0.184
Total bone mineral content (kg)[a]	2.18 (1.83-2.37)	2.10 (1.87-2.36)	1.90 (1.76-2.19)	2.06 (1.78-2.26)	0.482
Trunk fat mass (kg)[a]	6.73 (3.69-8.39)	9.02 (7.94-11.97)	9.16 (7.26-11.18)	8.76 (7.59-11.37)	<0.001
Limb fat mass (kg)[a]	6.11 (4.978.29)	8.04 (6.25-11.21)	8.04 (5.31-11.44)	6.36 (4.44-9.01)	0.016
TLFR[a]	0.91 (0.70-1.12)	1.18 (0.99-1.69)	1.14 (0.89-1.36)	1.47 (1.21-1.84)	<0.001
PTF (%)[a]	45.86 (38.35-51.17)	52.32 (46.91-59.46)	51.5 (44.03-56.52)	57.32 (49.05-61.84)	0.152
Android/gynoid (A/G) ratio[a]	0.58 (0.45-0.85)	0.51 (0.34-0.89)	0.60 (0.50-0.79)	0.67 (0.51-1.00)	0.504

[a]All non-normally distributed variable expressed as median (25-75th percentile). Abbreviations are as given in Table I

Table V. Binary logistic regression analysis showing factors that independently predict the occurrence of hypercholesterolaemia in patients with HIV infection

Variable	β	Exp(β)	*P*
Age	−0.012	0.988	0.743
Delta CD4 count	0.001	1.001	0.567
Total lean mass	−0.00001	0.9999	0.768
Truck fat mass	0.0004	1.0004	0.097
Total fat per cent	−0.091	0.913	0.345
PTF	0.041	1.042	0.044

Binary logistic regression was initially performed with all parameters which are likely to influence the occurrence of hypercholesterolaemia. Parameters with $P<0.2$ were included into the final model Exp(β): exponentiation of the β coefficient, change in odds ratio with 1 unit change in predictor variable; for categorical variablew immune reconstitution, patients with immune reconstitution were taken as the reference group; ART, anti-retroviral therapy; PTF, per cent trunk fat; BMI, body mass index; PBF, per cent body fat

suggested that increased inflammatory response to HIV stimulates homoeostatic response to stress at the cellular level, resulting in adverse impact on adipocytes metabolism, resulting in dyslipidaemia and abnormal body fat distribution.

Lipoatrophy was observed in 8.56 per cent patients in our study. Miller *et al*[22] have documented lipoatrophy/lipodystrophy in 15-50 per cent of patients with HIV infection. A previous study from India documented lipodystrophy in 46.1 per cent of 306 patients on ART[23]. In this study, the occurrence of lipodystrophy was associated with dyslipidaemia and fasting hyperglycaemia[23]. The large difference in occurrence of lipoatrophy/lipodystrophy in different studies may be explained by ethnic differences, heterogeneity in the profile of patients evaluated and different definitions used for assessment of lipoatrophy/lipodystrophy. In a previous study from south India, the occurrence of lipodystrophy was 60.7 per cent (22.7% with lipohypertrophy, 51.1% with lipoatrophy and 22.7% with mixed pattern) in 145 HIV patients on ART[4], which was much higher than our study. However, the patients in that study were more sick, had more severe disease with greater immunodeficiency and comorbidities as compared to our study[4].

Increased duration of ART use has been linked with lipoatrophy[24]. Patients with lipoatrophy in our study had increased dysglycaemia, IR and occurrence of MetS, which was in accordance with previous studies, which linked lipoatrophy with hypertension, diabetes, IR and cardiovascular disease[25,26]. Lipoatrophy was not a predictor of dyslipidaemia in patients with HIV infection in this study on regression analysis. Serum triglycerides were comparable in patients with lipoatrophy as compared to those without. Only serum TC and LDL-C were elevated in patients with lipoatrophy. Body composition parameters were not altered in patients with lipoatrophy as compared to those without. Several previous studies on children and adult with HIV infection have also not documented any relationship between lipoatrophy and dyslipidaemia[15,27,28]. The cause for this apparent lack of link between lipoatrophy and dyslipidaemia in HIV is not known. A few studies have suggested that it is lipodystrophy and not lipoatrophy, which is associated with dyslipidaemia[15,28]. Lipodystrophy is a spectrum disorder with lipoatrophy and lipohypertrophy being a part of it.

The limitation of this study was that skinfold thickness at different sites of the body and lipohypertrophy were not evaluated. The fact that per cent truck fat was not significantly different in patients with lipoatrophy as compared to those without lipoatrophy might explain its poor clinical utility as a predictor of dyslipidaemia. Strengths of this study include the use of the gold standard DEXA for the assessment of body fat distribution in HIV patients with stable immune function. Another limitations of this study was its cross-sectional nature. Control patients with HIV infection but not on ART were not evaluated. Further, healthy controls were not evaluated in this study. This study was based on data collected from one ART centre and hence needs to be replicated at other centres, before it can be generalized to the entire population of Indians living with HIV.

In conclusion, Indians patients with HIV infection with stable immune function showed high occurrence of dyslipidaemia, which has major public health implications related to long-term risk of atherosclerotic cardiovascular disease. Increased central adiposity (trunk FM) with loss of peripheral adiposity was found to be the most important predictor of dyslipidaemia in patients with HIV infection.

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Table VI. Clinical, anthropometric, metabolic and body composition profile of patients with HIV infection with lipoatrophy as compared to those without lipoatrophy

Parameter	Lipoatrophy present (n=22)	No lipoatrophy (n=235)	*P*
Age (yr)	37.22±6.32	37.28±7.58	0.379
Sex (male:female)	14:8	167:68	0.306
BMI (kg/m^2)	22.3±4.63	22.5±4.06	0.572
History of tuberculosis	7	73	0.586
History of immune reconstitution	8	61	0.218
NRTI use	17	184	0.449
NNRTI use	17	177	0.467
Protease inhibitors use	1	6	0.468
MetS	12	61	0.009
At least 1 lipid abnormality	20	183	0.118
Total cholesterol (mg/dl)[a]	207 (172-248)	185 (154-211)	0.008
Triglycerides (mg/dl)[a]	154 (125-189)	145 (177-196)	0.744
LDL-C (mg/dl)[a]	132 (100-166)	108 (80-131)	0.005
HDL-C (mg/dl)[a]	42 (36-46)	46 (38-52)	0.189
FBG (mg/dl)[a]	90 (86-110)	87 (82-94)	0.009
HOMA-IR[a]	2.04 (1.71-4.30)	1.76 (1.42-2.52)	0.009
QUICKI	0.34 (0.31-0.36)	0.35 (0.33-0.36)	0.100
HOMA-β[a]	121.4 (118.1-127.4)	121 (116-126)	0.613
Duration of HIV infection (months)[a]	58 (51-94)	56 (33-102)	0.741
Baseline CD4 count (pre ART) (cell/µl)[a]	179 (102-320)	187 (114-351)	0.195
CD4 count (6 months post ART) (cell/µl)[a]	329 (256-385)	293 (199-414)	0.626
Current CD4 count (cell/µl)	389 (277-695)	393 (265-528)	0.910
Delta CD4 count (cells/mm^3)	109 (37-242)	76 (0-200)	0.279
25OHD (ng/ml)[a]	20.05 (14.87-22.03)	18.10 (12.85-21.8)	0.368
Total fat mass (kg)[a]	16.22 (11.49-22.48)	15.36 (12.13-22.18)	0.261
Total fat per cent (%)	27.6 (19.3-36.8)	27.5 (22.15-36.42)	0.489
Total lean mass (kg)[a]	38.74 (31.34-46.91)	40.09 (33.35-47.53)	0.744
Total bone mineral content (kg)[a]	1.96 (1.81-2.24)	2.08 (1.80-2.28)	0.557
Trunk fat mass (kg)[a]	7.84 (5.58-9.63)	8.35 (6.65-10.47)	0.784
Limb fat mass (kg)[a]	6.71 (4.26-8.44)	6.72 (5.19-8.98)	0.562
TLFR[a]	1.14 (0.92-1.57)	1.17 (0.91-1.52)	0.466
PTF (%)[a]	50.88 (45.34-58.59)	50.99 (44.28-58.66)	0.890
Android/gynoid (A/G) ratio[a]	0.56 (0.47-1.01)	0.62 (0.49-0.85)	<0.001

[a]All non-normally distributed variable expressed as median (25-75th percentile). Abbreviations are as given in Table I

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