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ORIGINAL ARTICLE

Dyslipidemia in HIV Infected Children Receiving Highly Active Antiretroviral Therapy

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Abstract
Objective To assess the prevalence of dyslipidemia and lipodystrophy in Indian children receiving non-nucleoside reverse transcriptase inhibitor (NNRTI) based highly active antiretroviral therapy (HAART) and to determine the associated risk factors for the same.
Methods The present cross-sectional study was conducted at a Pediatric Clinic of a tertiary care teaching center in India, from May 2011 through December 2012. HIV infected children aged 5–15 y were enrolled if they did not have any severe disease or hospital admission within last 3 mo or receive any medications known to affect the lipid profile. Eighty-one children were on highly active antiretroviral therapy (HAART) for at least 6 mo and 16 were receiving no antiretroviral therapy (ART). Participants' sociodemographic, nutritional, clinical, and laboratory data were recorded in addition to anthropometry and evidence of lipodystrophy. Fasting lipid profile, apolipoprotein A1 and B levels were done for all the children.
Results Among the children on highly active antiretroviral therapy (HAART), 38.3 % had dyslipidemia and 80.2 % had lipodystrophy, while 25 % antiretroviral therapy (ART) naïve HIV infected children had dyslipidemia. No clinically significant risk factors could be identified that increased the risk of dyslipidemia or lipodystrophy in children on highly active antiretroviral therapy (HAART).
Conclusions There is a high prevalence of dyslipidemia and lipodystrophy in Indian children with HIV infection with an imminent need to establish facilities for testing and treatment of these children for metabolic abnormalities.

Keywords HIV infection · Dyslipidemia · Lipodystrophy · Highly active antiretroviral therapy

Introduction

With the advent of highly active antiretroviral therapy (HAART), the life expectancy of human immunodeficiency virus (HIV) infected patients has increased significantly over the last 2 decades [1–3], leading our focus to the long term effects of antiretroviral therapy (ART). Dyslipidemia and lipodystrophy syndrome with HIV infection, compounded by its therapy pose a special risk to the pediatric population in view of their early and subsequent life-long exposure to such agents [4, 5]. Although the literature is replete with studies and reviews of dyslipidemia and lipodystrophy syndrome in adult patients receiving HAART, the pediatric literature is relatively sparse [6, 7], especially in children receiving Non-nucleoside reverse transcriptase inhibitor (NNRTI) based HAART [8, 9]. A large multicentric study from Europe [10] reported a prevalence of dyslipidemia and body fat redistribution to be 38 % and 26 % respectively in children on HAART. While a study from India [9] reported a significantly higher prevalence of hypercholesterolemia in children on HAART compared to HIV infected ART naïve children but only 2 out of 52 children had features of lipodystrophy.

This study was carried out to find the prevalence of dyslipidemia and lipodystrophy in the HIV infected Indian children

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on NNRTI based HAART with an attempt to identify associated risk factors.

Material and Methods

HIV infected children aged 5–15 y, being followed up in the Pediatric Clinic of a tertiary care teaching center in North India were enrolled from May 2011 through December 2012. Ethical clearance was obtained from the Institute Ethics Committee and a written informed consent was taken from either of the parents/legal guardian. Eighty one children receiving HAART for at least 6 mo and 16 patients not receiving any ART were included if they did not have any severe disease or hospital admission within last 3 mo. All the patients were treated as per the National AIDS Control Organization (NACO) guidelines 2006 [11]. Patients receiving any other drug known to affect the lipid profile were also excluded. Demographic data including family history of dyslipidemia, data related to the HIV disease status and therapy were recorded. The WHO clinical and immunological staging of the HIV disease were also noted [12]. Anthropometric measurements were compared with the Centre for Disease Control (CDC) references and the mean 'z' scores for weight for age (WAZ), height for age (HAZ) and body mass index (BMI) (BMZ) were calculated using Epi info version 7.0.9.34 (CDC, Atlanta). Participants were evaluated for the evidence of fat redistribution (peripheral lipoatrophy and central lipohypertrophy) on a predesigned checklist. Dietary intake was documented by 24 h dietary record. Thereafter, calorie, protein and cholesterol intakes were derived using "Nutritive Value of Some Common Foods Charts" (National Institute of Nutrition, ICMR, Hyderabad) and were expressed in terms of percentage of recommended dietary allowance (RDA) for Indian children [13] of the same age and gender for protein and calories and as mg/kg for cholesterol.

Children were tested for lipid profile consisting of total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides (TG), very low density lipoprotein (VLDL) cholesterol and apolipoprotein A1 & apolipoprotein B after an overnight fasting of at least 8 h. The assays used were as follows: Total cholesterol–Randox Trinder based CHOD PAP calorimetric end point assay, CH 3810; Triglycerides–Randox Trinder based GPO PAP calorimetric end point assay, TR 2823; HDL-cholesterol–Randox- liquid ready to use two point kinetic assay, CH 3811; Apolipoprotein A1 and apolipoprotein B–Randox Immunoturbidometric assay, HITACHI.

VLDL cholesterol was calculated by the formula VLDL = TG/5 and LDL cholesterol was calculated by using Friedewald equation [LDL = total cholesterol - (HDL + VLDL)] [14].

Dyslipidemia was defined according to the American Academy of Pediatrics National Cholesterol Education Programme (NCEP) [15] and if a patient had any of the following, a label of dyslipidemia was assigned; Hypercholesterolemia: Total plasma cholesterol >200 mg/dl; Hypertriglyceridemia: Triglyceride >150 mg/dl; LDL cholesterol >130 mg/dl; HDL cholesterol <35 mg/dl.

Lipodystrophy included either lipoatrophy (Reduced fat in peripheral areas, such as arms, legs and buttocks and relatively prominent muscles and veins) or lipohypertrophy (increased fat in the abdominal region, dorsal gibbosity, gynecomastia and increased breast size in adolescents and females) or a combination of the two. Fat redistribution were scored [16] separately for limbs, face, buttocks, abdomen, thorax and neck on a scale of 0 to 3 [0 = absent, 1 = mild (noticeable on close inspection), 2 = moderate (readily noticeable by patient/physician), 3 = severe (readily noticeable to a casual observer)].

Data were collected on a structured performa and managed on Microsoft Excel sheet. The data were analyzed using Stata 9 software (STATA Corp, College Station, TX). The baseline data was described as mean ± SD. The prevalence of dyslipidemia and lipodystrophy were calculated along with 95 % confidence intervals. Based on the results, there were 2 groups of children- those with dyslipidemia and those without; these 2 groups were compared to determine the factors associated with dyslipidemia. Similar analysis was performed for factors associated with lipodystrophy. Student-t test and chi square test were used for continuous and categorical data respectively. No logistic regression multivariate analysis was performed in view of small sample sizes.

The numbers of ART naïve HIV infected children were low, but an attempt was made to compare the prevalence of dyslipidemia between the patients receiving HAART and ART naïve patients.

Results

The baseline characteristics of the 81 children, who received HAART for at least 6 mo and 16 ART naïve patients, are depicted in Table 1. There were only 2 children in the HAART group having a family history of dyslipidemia and none of the participants were from the upper socio-economic strata. Most of the patients acquired their infection by vertical mode of transmission.

Lipid profile parameters, apolipoprotein A1 and apolipoprotein B values of ART naïve children were compared with those of the children receiving HAART (Table 2). Though all the measured parameters were found to be higher in children receiving HAART, the difference was statistically significant only for total cholesterol and apolipoprotein A1.

Thirty one out of 81 children on HAART had at least one abnormal parameter in their lipid profile. Thus, the prevalence

Table 1 Baseline characteristics of HIV infected children receiving HAART and ART naïve

Baseline characteristics	Patients on HAART (n = 81)	ART naive patients (n = 16)
Mean age in months (SD)	128.4 (32.3)	105.69 (28.7)
Boys	55 (68 %)	14 (87.5 %)
Positive family history of dyslipidemia	2 (2.4 %)	0
Socio-economic status (Modified Kuppuswamy scale) (n = 63)		
Upper	0	0
Upper-middle	25 (30.8 %)	6 (37.5 %)
Lower-middle	18 (22.2 %)	5 (31.3 %)
Upper-lower	18 (22.2 %)	4 (25 %)
Lower	2 (2.47 %)	1 (6.2 %)
Age at diagnosis [Mean (SD) months]	68.4 (36.4)	73.4 (33.5)
Mode of transmission		
Vertical	67 (82.7 %)	12 (75 %)
Parenteral	2 (2.4 %)	2 (12.5 %)
Not known	12 (14.8 %)	2 (12.5 %)
Nutritional status		
WAZ [Mean (SD)]	−1.8 (1.4)	−1.8 (2.6)
HAZ [Mean (SD)]	−1.6 (1.3)	−1.3 (2.1)
BMZ [Mean (SD)]	−1.1 (1.4)	−1.0 (1.3)
Nutritional intake		
Mean of calorie intake/day [% RDA (SD)]	67.6 (20.3)	72.56 (26.82)
Mean of protein intake/day [% RDA (SD)]	117.5 (43.5)	114.17 (36.8)
Mean of cholesterol intake/day [mg/kg (SD)]	6.11 (2.14)	5.88 (2.78)
Clinical stage (WHO) at time of diagnosis		
I	35 (43.2 %)	12 (75 %)
II	29 (35.8 %)	4 (25 %)
III	17 (21 %)	0
IV	0	0
Current clinical stage (WHO)		
I	44 (54.3 %)	14 (87.5 %)
II	31 (38.2 %)	2 (12.5 %)
III	6 (7.4 %)	0
IV	0	0
Current CD 4 count [Mean (SD)/mm^3]	900.4 (573.1)	661.5 (431.5)
Current CD 4 count		
≥ 500/mm^3	59 (72.8 %)	10 (62 %)
200–499/mm^3	21 (25.9 %)	3 (19 %)
< 200/mm^3	1 (1.2 %)	3 (19 %)
Duration of ART [Mean (SD) months]	54.8 (32.0)	NA
ART combination		NA
Stavudine + Lamivudine + Nevirapine	57 (70.3 %)	
Ziduvudine + Lamivudine + Nevirapine	12 (14.8 %)	
Stavudine + Lamivudine + Efavirenz	11 (13.5 %)	
Ziduvudine + Lamivudine + Efavirenz	1 (1.2 %)	
Duration of current ART regimen [Mean (SD) months]	50.3 (33.3)	NA

of dyslipidemia in HIV infected children on HAART was estimated to be 38.3 % [95 % CI: 28.4 to 49.2 %]. While the prevalence of dyslipidemia in HIV infected-ART naive patients was 25 % (95 % CI: 3.8 to 46.2 %) (Table 3). Most common dyslipidemia in patients on HAART was hypertriglyceridemia followed by hypercholesterolemia, while in ART naïve patients hypercholesterolemia was the most prominent abnormality (Table 3).

Factorial analysis failed to show any statistically significant correlation between the use of HAART and prevalence of dyslipidemia in the studied population (p 0.4).

The prevalence of lipodystrophy in HAART treated children was 80.2 % (CI: 71.5–88.9 %), with isolated lipoatrophy being the most common type (60.5 %) followed by combined type (11.1 %). No significant association was found between biochemical dyslipidemia and clinical lipodystrophy (p 0.26).

Table 2 Fasting lipid profile, apolipoprotein A1 and apolipoprotein B levels of HIV infected children receiving HAART and ART naïve

Lipid profile	HAART (n = 81) [Mean (SD)]	ART naive (n = 16) [Mean (SD)]	P value
Total cholesterol (mg/dl)	162.1 (32.9)	142.4 (33)	**0.03**
Triglycerides (mg/dl)	115.9 (59.8)	96 (32.5)	0.20
LDL (mg/dl)	91.0 (29.4)	80.2 (27.3)	0.18
HDL (mg/dl)	47.4 (8.9)	42.9 (6.8)	0.05
VLDL (mg/dl)	23.1 (11.9)	19 (6.6)	0.19
Apolipoprotein A1 (mg/dl)	121.4 (32.2)	103.2 (25.3)	**0.04**
Apolipoprotein B (mg/dl)	72.3 (17.9)	66.2 (15.2)	0.20

The statistically significant p values of the compared observations are in bold

On comparing children with and without dyslipidemia, only the mode of HIV transmission was found to be significantly different in children having dyslipidemia from those with no dyslipidemia (Online Resource 1). Among the risk factors for lipodystrophy, only current clinical stage of the disease was found to be statistically relevant (Online Resource 2). Even nutritional intakes were not found to affect the prevalence of dyslipidemia or lipodystrophy.

Discussion

In this cross sectional study, the authors analyzed 81 HIV infected children, aged 5–15 y, on NNRTI based HAART and also 16 HIV infected ART naive patients of the same age group to estimate the prevalence of dyslipidemia, lipid redistribution and their associated risk factors. The present study discloses a high prevalence of dyslipidemia [38.3 % (95 % CI 28.4–49.2)] and lipodystrophy [80.2 % (95 % CI 70.3–87.5)] in patients receiving HAART.

There are wide variations in the prevalence rate of dyslipidemia among HIV-infected children on ART [6–10, 17]. The authors found that, the children receiving HAART had hypertriglyceridemia as the most frequent abnormality (22.2 %) of lipid profile, followed by hypercholesterolemia (14.8 %). The results of abnormal lipid profile in the index study are consistent with the European Pediatric Lipodystrophy Group study [10]. The pattern of lipid profile abnormalities is also partly consistent with the findings of other studies [6–8] done in pediatric population, which reveal the most consistent abnormality to be hypertriglyceridemia. But the second most common abnormality observed in these studies *i.e.*, low HDL was the least prevalent dyslipidemia defining condition in the index study. A plausible explanation could be the more frequent use of nevirapine based regimen in the index population which is postulated to increase the HDL [18].

A pediatric study published from India [9] had shown a lower prevalence of hypertriglyceridemia (16 %). Mean levels of triglyceride [115.9 (± 59.8) *vs*. 97.2(± 56.3)] and HDL levels [47.4 (± 8.9) *vs*. 41.0(± 9.3)] were also different; in this study, 22 of 25 patients on ART received stavudine based regimen. But another prospective Asian study from Thailand [17] had shown abnormal lipid parameters similar to the index study.

On comparing with ART naïve children, the percentage of children having different abnormal parameters in the lipid profile was not significantly different from those receiving HAART. Prevalence of dyslipidemia was also not statistically correlated with the use of HAART.

The prevalence of lipodystrophy was alarmingly high in the studied population (80.2 %). This unusually high prevalence is inconsistent with the previous studies in the pediatric population which depicted a prevalence ranging from 10 % to 65 % [9, 10, 19]. The most plausible explanation could be high prevalence of undernutrition in these children, evident by the nutritional

Table 3 Dyslipidemia in HIV infected children receiving HAART and ART naïve

Lipid profile	Patients on HAART (n = 81) [n (%)]*	ART naïve Patients (n = 16) [n (%)]*
Total cholesterol (>200 mg/dl)	12 (14.8)	2 (12.5)
Triglyceride (>150 mg/dl)	18 (22.2)	1 (6.3)
LDL (>130 mg/dl)	8 (9.9)	1 (6.3)
HDL (<35 mg/dl)	3 (3.7)	0
Dyslipidemia	**31 (38.3)**	**4 (25)**
95 % CI	**(28.4 to 49.2)**	**(3.8 to 46.2)**

The primary objectives of the study are in bold

*Some children had more than one abnormality in the lipid profile

status assessment which revealed their mean weight for age, height for age and BMI for age 'z' scores as −1.8, −1.6 and −1.1 respectively, which could contribute to the lipoatrophy observed. Again the nutritional intake evaluation revealed calorie intakes to be clearly inadequate with mean calorie intake being only about 68 % of RDA although protein intakes were good (mean protein intake 118 % of RDA). This discordance in calorie and protein intakes can give rise to disturbances in redistribution of body mass.

No statistically significant association was found between dyslipidemia and lipodystrophy. This again is not in accord with the previous studies [10], possibly due to a smaller sample size. Caution is needed in describing the association of high prevalence of lipodystrophy seen in HIV infected children on HAART with dyslipidemia, as more prospective studies are needed to gain a better understanding of the complex relationship.

Failure to identify any strong risk factors for dyslipidemia and lipodystrophy could be attributed to the small number of subjects in each group.

Some limitations were noted and must be acknowledged in this study. The number of study subjects was not high, thereby limiting power of the analysis. Secondly, associations could not be said to be causal because of the cross sectional nature of the study. An optimal study design will be to evaluate these parameters in a cohort of children started on HAART. There was a lack of standardized definition of lipodystrophy in children. The authors used a classification that was also used in an Indian study [9].

Despite these limitations, assuming that the sample of patients studied is representative, the present results indicate a high prevalence of dyslipidemia and lipodystrophy and subsequent possible risk for the development of cardiovascular disease in a significant proportion of children with HIV/AIDS on HAART. Cardiovascular disease is currently the second most frequent cause of death (after cancer) among HIV-positive subjects (adults) in areas of the world where HAART is widely available [20]. Many studies have demonstrated an association between HAART and increased risk of coronary events when compared to the general population. This may be related to dyslipidemia and to the duration of HAART exposure [21–24]. In multivariate analyses, for every mmol/L increase in total cholesterol, the relative risk of myocardial infarction increased by a factor of 1.26 *i.e.*, 26 % [21].

Furthermore, as most of the patients are unaware of their lipid abnormalities, the findings of this study underscore the need to adopt strategies to routinely evaluate and modify the risk of cardiovascular diseases in this population and an urgent need for formulation of guidelines in par with the adult guidelines as the duration of these metabolic abnormalities are substantially longer in children.

Contributions SKK and RL conceived the concept and designed the study and also were the experts of HIV treatment. AM did the literature search, acquired the clinical data and laboratory samples and drafted the manuscript. RL conducted the laboratory tests. AM helped in analysis of data and writing the manuscript. All authors approved the final manuscript. SKK will act as guarantor for this paper.

Conflict of Interest None.

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