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Dyslipidaemia and insulin resistance in vertically HIV-infected children and adolescents

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ABSTRACT

This cross-sectional study determined the influence of antiretroviral therapy (ART) on the lipid profile and insulin sensitivity of 119 perinatally HIV-infected Brazilian patients aged 6–19 years. Inadequate high-density lipoprotein cholesterol (HDL-c) concentrations were observed in 81.4% of patients. High concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were found in 33.9%, 9.7% and 35.6% of patients, respectively. There were statistically significant differences in mean concentrations of TC ($P = 0.004$), HDL-c ($P = 0.015$) and LDL-c ($P = 0.028$) among children (<10 years), early adolescents (10–14 years) and late adolescents (15–19 years). Children presented the highest mean concentrations of TC and LDL-c, and patients in late adolescence presented the lowest concentrations of HDL-c. Insulin sensitivity, assessed by the Homeostasis Model Assessment (HOMA) index, was diagnosed in 16.7% of patients, with a statistically higher proportion ($P = 0.034$) of insulin-resistant children (33.3%) compared with adolescents (12.5%). There was a statistically significant association between TG concentrations and use of ART regimens containing protease inhibitors (PI) ($P = 0.0003$). Children presented a higher prevalence of insulin resistance and dyslipidaemia compared with adolescents, suggesting that ART, especially PIs, may lead to metabolic complications.

1. Introduction

Antiretroviral therapy (ART) has dramatically increased the longevity of HIV-infected children and adults. Highly active antiretroviral therapy (HAART), a combination of at least three drugs, has been associated with substantial reductions in morbidity and mortality.[1,2] HAART regimens are based on either protease inhibitors (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTI) combined with nucleoside reverse transcriptase inhibitors (NRTI).[3]

Despite the benefits of HAART on the clinical and nutritional condition of patients, a considerable number of patients present complications such as body fat redistribution (lipodystrophy), dyslipidaemia and insulin resistance.[4,5] According to Krause et al.,[5] lipodystrophy may be common in some HIV-infected children on ART.

Adverse effects have been associated with HAART, particularly with the use of PIs, in paediatric and adult patients.[6] HAART regimens with PIs are linked to metabolic abnormalities, including increased levels of atherogenic lipoproteins, insulin resistance and diabetes.[7] Possible risk factors for the development of morphological and metabolic disturbances among children receiving ART are pubertal development during PI therapy, the virological response to HAART,[8] severity and duration of HIV

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infection, duration and type of ART, suppression of viraemia, and lipid abnormalities before the introduction of ART.[9]

It has been suggested that metabolic abnormalities are of great concern, especially for vertically HIV-infected children since ART is introduced early in life.[4] A scientific statement from the American Heart Association noted that 20–50% of HIV-infected children treated with PIs developed metabolic abnormalities, most commonly increased concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c).[10]

As far as we know, there is no epidemiological study in Brazil assessing the side effects of ART in vertically HIV-infected children. Therefore, the objective of this study was to determine the influence of antiretrovirals on the lipid profile and insulin sensitivity of HIV-infected children and adolescents.

2. Materials and methods

A cross-sectional descriptive study involving 119 perinatally HIV-infected children and adolescents was undertaken between August and December 2007 at the HIV/AIDS outpatient clinic of the Institute of Child Health, University of São Paulo (São Paulo, Brazil). Patients aged 6–19 years (schoolchildren and adolescents) who were vertically HIV-infected and whose caregivers signed informed consent were included in the study.

The HIV/AIDS clinic of the Institute of Child Health is one of the three largest reference centres for treatment of paediatric patients with HIV/AIDS in São Paulo city, receiving patients not only from this city but also from other regions of the country.

In Brazil it is an obligation of the State to distribute treatment universally and free of charge since 1996,[11] and all the health services are obligated to follow the guidelines defined by the Health Ministry. Based on the information specified above, the sample can be considered as representative of the HIV-infected Brazilian paediatric population.

Data were collected by physical examination, by a questionnaire given to the patient's caregiver and from medical records. Information on demographic, obstetric, clinical and anthropometric variables, medical history including the use of antiretrovirals over the last 3 years, and recent biochemical tests [TC, high-density lipoprotein cholesterol (HDL-c), LDL-c, triglycerides (TG), glucose and insulin] was obtained. The recall for medical history was limited to the last 3 years to ensure that patients had access to all classes of agents that characterise ART.

Lipids and glucose concentrations were determined using a Roche/Hitachi 917 system (Roche Diagnostics GmbH, Mannheim, Germany) by colorimetric enzymatic methods. LDL-c was calculated according to the equation described by Friedewald et al.[12] TC and fractions as well as TGs were classified according to the recommendations of the Brazilian Society of Cardiology[13] (TC <150 mg/dl; HDL-c ≥45 mg/dl; LDL-c <100 mg/dl; and TGs <100 mg/dl).

Insulin was determined by an immunofluorometric assay using an Auto DELFIA insulin kit B080-101 (Wallac, Turku, Finland). The Homeostasis Model Assessment (HOMA) described by Matthews et al.[14] was used to determine insulin sensitivity. A HOMA value ≥2.5 for children and ≥4.0 for adolescents indicated the presence of insulin resistance.[15]

CD4 values were used to assess the clinical condition of the patient according to the reference parameters of the Brazilian Health Ministry[16] for the paediatric population. Results were grouped into three categories: no immunological suppression (≥500 cells/mm^3); slight suppression (350–499 cells/mm^3); and moderate/severe immunological suppression (≤349 cells/mm^3).

The parameters included in this study were: time of disease diagnosis; duration of ART; classes of antiretrovirals used over the last 3 years; CD4 count; lipid profile (TC, HDL-c, LDL-c and TGs); and the HOMA index.

Patients were grouped according to the WHO's definition of childhood (<10 years of age), early adolescence (10–14 years) and late adolescence (15–19 years).[17]

Statistical analysis was performed using Stata 9 software (Stata Corp., College Station, TX, USA). Mean, median, SD, and minimum and maximum values were assessed for the investigated variables. ANOVA was used to compare means of three or more variables with a normal distribution, and the Kruskal–Wallis test was used to compare means of variables with a non-normal distribution. The non-parametric Wilcoxon (Mann–Whitney) test was used to compare means of two variables with a non-normal distribution. Correlations were assessed by Spearman's non-parametric test. The level of significance was set at $P < 0.05$.

3. Results

All of the 120 eligible patients seen at the HIV/AIDS outpatient clinic of the Institute of Child Health were invited to participate in the study; only one eligible patient refused to participate.

Thus, the final sample comprised 119 children and adolescents aged 6–19 years. The sociodemographic, clinical and nutritional characteristics of the patients are summarised in Table 1.

The mean duration of exposure to antiretrovirals (8.6 years) was high owing to the fact that patients were infected by vertical transmission.

Some degree of immunodeficiency was found in 51.3% of subjects (Table 1) and only three patients (2.5%) had not used any medication over the last 3 years. It was not possible to collect the medication history of one patient because his record was not available at the institution.

The regimens adopted for 115 patients were: A (double therapy; two NRTIs); B (triple therapy; two NRTIs and one PI); C (triple therapy; two NNRTIs and one PI); D (triple therapy; two NRTIs and one NNRTI); and E (alternative therapy; one drug of each class, i.e. one PI, one NRTI and one NNRTI). Double therapy was adopted for 29 patients (25.2%), triple therapy for 63 patients (54.8%) and alternative therapy for 23 patients (20.0%).

Almost all patients (99.1%) on ART used NRTIs, 55.7% received PIs and 40.0% NNRTIs.

Mean concentrations of TC, LDL-c and TGs were adequate, but the mean concentration of HDL-c was low (Table 1).

Table 1
Characteristics of the 119 vertically HIV-infected children and adolescents included in the study

Variable	*n*	%	Mean ± SD[a]
Age (years)			11.9 ± 2.9
6–9	26	21.8	
10–14	67	56.3	
15–19	26	21.8	
Total	119	100.0	
Per capita income (Brazilian Real[b]) [median (range)]			266.66 (50–3750)
50–175	38	34.5	
182–350	37	33.6	
375–3750	35	31.8	
Total	110[c]	100.0	
Gender			
Male	58	48.7	
Female	61	51.3	
Total	119	100.0	
Nutritional status (BMI/age[d])			
Malnourished	5	4.2	
Eutrophic	96	80.7	
Overweight	15	12.6	
Obese	3	2.5	
Total	119	100.0	
Homeostasis Model Assessment			
Insulin-resistant	15	16.7	
Non-insulin-resistant	75	83.3	
Total	90[c]	100.0	
Total cholesterol (mg/dl)			154.7 ± 39.8
Elevated	40	33.9	
Normal	78	66.1	
Total	118[c]	100.0	
High-density lipoprotein cholesterol (mg/dl)			35.7 ± 10.1
Low	92	81.4	
Normal	21	18.6	
Total	113[c]	100.0	
Low-density lipoprotein cholesterol (mg/dl)			93.9 ± 28.5
Elevated	11	9.7	
Normal	102	90.3	
Total	113[c]	100.0	
Triglycerides (mg/dl)			121.4 ± 75.5
Elevated	42	35.6	
Normal	76	64.4	
Total	118[c]	100.0	
Degree of immunodeficiency (CD4 cells/mm^3)			
Without suppression (≥500)	58	48.7	
Slight (350–499)	28	23.5	
Moderate/severe (≤349)	33	27.7	
Total	119	100.0	

[a] Data are mean ± SD unless otherwise stated.
[b] US$1 = R$1.89 Brazilian Real.
[c] Some patients did not perform the biochemical tests or answer the questionnaire.
[d] Body mass index (BMI) evaluated through percentiles according to the WHO.[31]

There was a statistically significant difference between mean TC concentrations in children, adolescents aged 10–14 years (early adolescence) and adolescents aged 15–19 years (late adolescence) ($P = 0.004$). The mean ± SD concentrations of TC (mg/dl) observed in the three groups of patients were 173.1 ± 36.8, 154.3 ± 41.2 and 136.9 ± 30.9, respectively (Figure 1A).

There was a statistically significant difference in mean HDL-c in the three groups of patients ($P = 0.015$). The mean ± SD HDL-c concentrations (mg/dl) obtained in children, early adolescents and late adolescents were 39.8 ± 8.5, 34.8 ± 10.0 and 33.2 ± 11.1, respectively (Figure 1C).

There was a statistically significant difference in mean LDL-c in the three groups of patients ($P = 0.028$). The mean ± SD LDL-c concentrations (mg/dl) obtained in children, early adolescents and late adolescents were 104.5 ± 26.4, 93.1 ± 30.1 and 83.5 ± 21.5, respectively (Figure 1B).

There was no statistically significant difference in mean concentrations of TGs among children and early and late adolescents ($P = 0.689$).

There were more children with insulin resistance (33.3%) than adolescents (12.5%) ($P = 0.034$).

The mean ± SD TC concentration in patients without immunological suppression (170.5 ± 41.3 mg/dl) was higher than in patients with slight (138.9 ± 29.4 mg/dl) and moderate/severe (140.1 ± 34.4 mg/dl) immunological suppression ($P < 0.001$).

Patients without immunological suppression had a mean ± SD HDL-c concentration of 39.1 ± 9.2 mg/dl compared with 31.2 ± 6.9 mg/dl in patients with slight immunological suppression and 33.0 ± 12.0 mg/dl in patients with moderate/severe immunological suppression ($P < 0.001$). The mean LDL-c concentration in patients without immunological suppression (102.5 ± 29.4 mg/dl) was higher than in patients with slight (86.5 ± 21.4 mg/dl) and moderate/severe (84.1 ± 28.1 mg/dl) immunological suppression ($P = 0.002$).

The duration of ART was not statistically different in patients with or without alterations in their lipid profile ($P > 0.05$).

To evaluate the relationship between the biochemical parameters investigated and the classes of antiretrovirals, the regimens were separated into PI-containing therapy (B, C and E) and non PI-containing therapy (A and D), as mentioned previously. The number of patients in each group was 64 (55.7%) and 51 (44.3%), respectively.

There was a statistically significant association between mean serum TG concentrations and antiretroviral regimens ($P = 0.0003$). Users of PI-containing regimens had the highest mean ± SD for TGs (144.3 ± 11.1 mg/dl) compared with users of non PI-containing regimens (95.1 ± 6.1 mg/dl). Thus, the regimens most associated with disturbances in mean TG concentrations were those with a PI (B, C and E).

Although HDL-c concentration was not statistically associated with the different types of antiretroviral regimens ($P > 0.05$), users of PI- and non-PI-containing therapy showed low mean concentrations of this lipoprotein.

4. Discussion

Patients included in this study used antiretrovirals on average for 8.6 years, demonstrating that they had started therapy at a very young age. This is a very concerning issue since many publications have demonstrated that despite the clinical and nutritional benefits of ART, it may lead to complications such as lipodystrophy and metabolic disturbances.[6,18–22] Therefore, the paediatric population is probably exposed to a higher risk of these complications

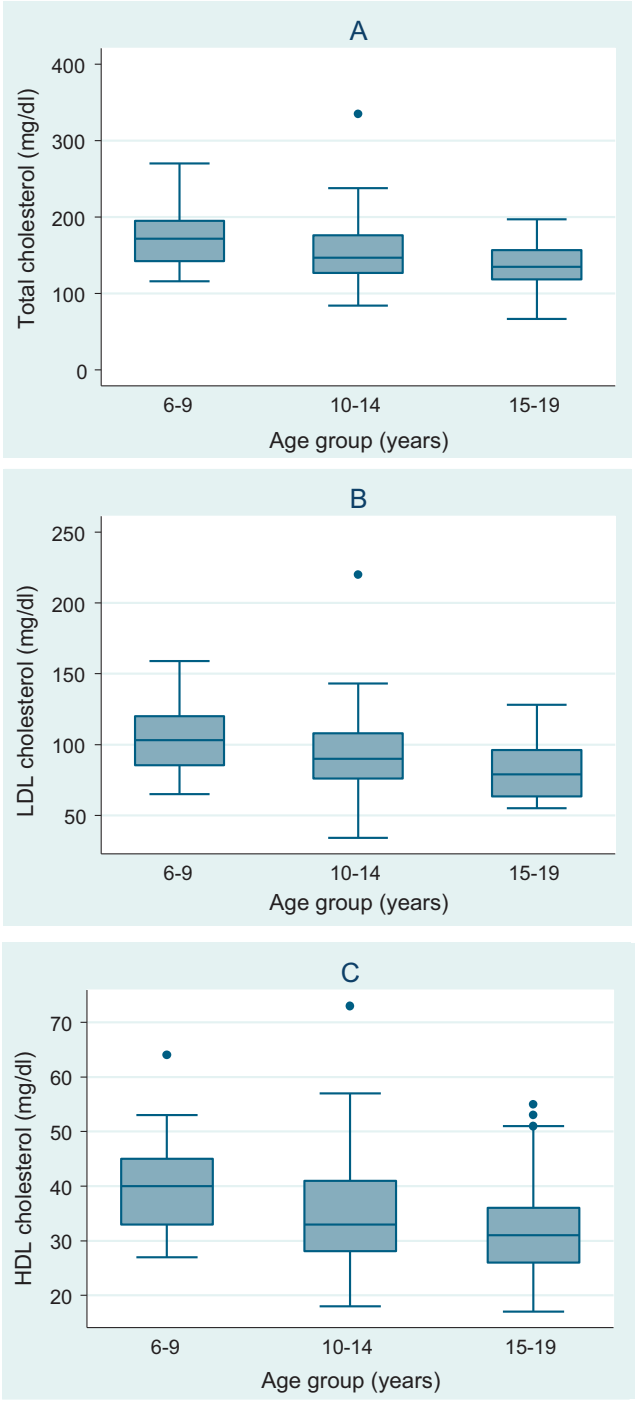

Figure 1. Box plots indicating mean ± SD concentrations of (A) total cholesterol (TC), (B) low-density lipoprotein cholesterol (LDL-c), and (C) high-density lipoprotein cholesterol (HDL-c) in vertically HIV-infected children and adolescents. ANOVA: TC, $P = 0.004$; LDL-c, $P = 0.028$; and HDL-c, $P = 0.015$. Dots indicate outlier values.

considering that use of antiretrovirals is for a longer period of time.[4]

Some authors have suggested that medication-related adverse effects are a barrier to treatment adherence.[23] Considering that the disease is chronic and that antiretrovirals are employed for prolonged periods, non-adherence to therapy may be one of the greatest public health challenges associated with the management of HIV/AIDS.[23]

In this study, a few individuals had not received antiretrovirals over the past 3 years. The most recommended regimen in the HIV/AIDS outpatient clinic of the Institute of Child Health was triple therapy with NRTI and

PI, which are the agents most frequently responsible for nutritional disturbances.[6,18,20,21,24]

Besides disturbances in the lipid profile, glucose metabolism can also be altered in HIV patients.[7] This study identified cases of insulin resistance mainly in children. McComsey and Leonard[1] noted that the mechanism by which patients receiving HAART develop insulin resistance has not been totally elucidated.

One of the most alarming findings in this study was the elevated number of patients with reduced HDL-c concentrations. Miller et al.[25] observed that HIV-infected children had a higher concentration of TGs and a lower concentration of HDL-c compared with controls. These authors suggest that HIV-infected children have adverse cardiac risk profiles and that ART has a significant influence on these factors. Aldrovandi et al.[21] identified a high prevalence of lipid abnormalities and evidence of insulin resistance in vertically HIV-infected children and youths using PIs, factors that may accelerate the lifetime risk of cardiovascular disease. Carter et al.[18] also considered that adverse effects derived from PI-containing regimens may expose children to a higher risk of cardiovascular diseases.

Alterations in the lipid profile were more frequent in children than adolescents, indicating that the youngest are more vulnerable to the effects of the disease and its associated therapy. Jaquet et al.[26] observed an increased frequency (23%) of dyslipidaemia in HIV-infected children without lipodystrophy, suggesting that hypertriglyceridaemia and/or hypercholesterolaemia could reflect changes in adipose tissue that precede fat redistribution. Torres et al.[24] also reported that disturbances in the lipid profile could precede the development of clinical alterations. Taylor et al.[8] observed that 16% of children receiving long-term PI therapy with consistently high lipid levels developed physical signs of fat redistribution with time. Kim et al.[27] suggested that the presence of lipoatrophy may be a marker for metabolic abnormalities, including dyslipidaemia.

PI-containing therapy may have a direct influence on the lipid profile by inhibiting an LDL-c receptor-related protein that blocks the uptake of lipoproteins and their metabolism or increases apolipoprotein B metabolism. Such therapy might also stimulate very-low-density lipoprotein (VLDL) cholesterol synthesis, induce adipogenesis and lower the expression of insulin receptors on adipocytes, consequently increasing the release of free fatty acids and inducing the hepatic production of lipoproteins.[25] Although studies point to a relationship between disturbances in the lipid profile and lipodystrophy, as far as we know there is no research confirming cause and effect.

Aldrovandi et al.[21] noticed that children/youths on PI-containing regimens had a higher prevalence of abnormal lipid profiles compared with HIV-negative subjects. Altered levels of TC (>200 mg/dl), LDL-c (>130 mg/dl), HDL-c (<35 mg/dl) and TGs (>130 mg/dl) were found in 29%, 19%, 10% and 52%, respectively. Aurpibul et al.[19] observed 11–12% dyslipidaemia in HIV-infected children receiving HAART. Ene et al.[28] and Beregszaski et al.[22] found metabolic abnormalities (hyperlipidaemia or insulin resistance) in 38.6% and 42.7% of HIV-infected children, respectively. Similar to our study, the authors cited above have observed metabolic disturbances in HIV/AIDS children and adolescents. However, it is difficult to compare these results considering that the cut-off points utilised for the different biochemical parameters are different.

The highest means of TC and LDL-c were observed in patients without immunological suppression. These findings may be explained by the association between antiretrovirals and better disease control. Taylor et al.[8] suggested that prolonged viral suppression may be a marker of maximal adherence and/or favourable pharmacokinetic parameters, which indicates maximal sustained exposure to ART. Thus, it is important to evaluate adherence and adverse effects among patients with long-term exposure to HAART, especially when ART is initiated early in life.

The occurrence of dyslipidaemia in HIV-infected children and its association with PI use have been shown in some studies.[5,20,21] Bockhorst et al.[29] observed insulin resistance and dyslipidaemia in HIV-infected children. According to these authors, all patients were receiving PI.

In the current study, there was no relationship between duration of ART and the lipid profile, similar to the results obtained by Ene et al.[28] However, the patients probably had a similar time of exposure to ART considering that this therapy became universally accessible after 1996.[11]

According to the present results use of a PI can alter the lipid profile, since users of PI-containing regimens had the highest means for TGs. Taylor et al.[8] noticed that when PI-containing regimens were introduced for patients aged <15 years they generally developed dyslipidaemia within the first year of therapy and maintained elevated lipid levels.

Some of the limitations of the study include lack of information regarding the use of antiretrovirals during the whole period of infection since the patients were born, lack of dietary intake data and exclusion of very young children in the sample. It is also important to point out that this type of study (cross-sectional) does not allow for causality assertions.

The 119 children included in the study probably characterise a sample of patients with long exposure to antiretrovirals. Matida et al.[11] noted that since 1998 there has been a significant reduction in mother-to-child transmission and an increase in survival of the HIV Brazilian paediatric population. According to these authors, the majority of Brazilian children and adolescents with HIV/AIDS receive long-term ART.

Despite some authors demonstrating that puberty appears to be the time when HIV-infected patients are most likely to develop metabolic disturbances,[22,30] in this study children had a better immunological condition and a higher prevalence of insulin resistance and dyslipidaemia than adolescents. Diagnosis of insulin resistance through HOMA is limited and the results of previous studies suggest that it is relatively uncommon in HIV-infected children on ART.[30] The main findings of this study may suggest that antiretrovirals, especially PIs, are efficient in the control of the disease but can lead to metabolic complications, especially in children. Another possible reason to explain this outcome is that the children evaluated in this study were exposed to the new antiretroviral agents early in life, which can have a different impact than when the first exposure

occurred later in life. Moreover, the medical team always discussed other options of medical therapy when dyslipidaemia was diagnosed. In this case, changes in the agents employed, when possible, as well as in the diet and lifestyle habits were advised. Thus, the benefits from these interventions were probably more evident among adolescents.

We advise the development of large epidemiological longitudinal studies involving HIV-infected children and adolescents to identify effective interventions to manage and prevent metabolic disturbances, in order to ensure better quality of life for these patients. It is also necessary to provide validated methods for diagnosing morphological and metabolic disturbances involved in the lipodystrophy syndrome among the HIV/AIDS paediatric population.

Authors' contributions: LCR designed the study protocol, collected data, participated in the statistical analysis and interpretation of data, and undertook the main writing of the paper; PHCR secured funding, designed the study protocol, facilitated data collection, and participated in the interpretation of data and writing of the paper; HHSM assisted in locating the study areas, co-ordinated data collection, and participated in the interpretation of data and writing of the paper; SBA collected data, and participated in the interpretation of data and writing of the paper. All authors read and approved the final manuscript. LCR is guarantor of the paper.

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