Original Article

Highly Active Antiretroviral Therapy Related Changes in Renal and Liver Functions in HIV Infection

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Alterations in liver and renal functions are among the complications of HIV infection, and highly active antiretroviral therapy (HAART) has been implicated. The combined nephrotoxic and hepatotoxic effects of HAART were determined by assessing the renal and liver functions of HIV sero-positive subjects on HAART in a tertiary hospital. Liver enzymes activities; Aspartate and alanine aminotransferases (AST & ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and renal function parameters; uric acid and creatinine clearance were determined in sera of 90 consenting subjects comprising of 30 HIV seropositive subjects on HAART, 30 HAART naive and 30 apparently healthy HIV sero-negative controls using colorimetric methods. CD_4^+ T cell count was done by flow cytometry while estimated glomerular filtration rate (eGFR) was determined by calculation. Anthropometric data and socio demographic information were obtained. Data was analyzed using ANOVA, LSD post hoc and Pearson's correlation at $p<0.05$. The body mass index and CD_4^+ T cell count were significantly higher and AST, ALT, LDH and uric acid levels lower in HIV sero-negative controls compared to HIV on HAART and HAART naïve subjects. HIV on HAART had higher AST, ALT, GGT and LDH activities compared to HAART naïve. A significant negative correlation ($r = -0.881$, $p = .000$) was observed between uric acid and CD_4^+ T cell count in HAART naïve subjects only. HIV infection and HAART is associated with low grade hepatotoxicity but no impairment of renal functions in the population studied.

Key words: HIV, HAART, liver enzymes, renal function

Introduction

Long-term viral suppression, decrease in opportunistic infections, preservation of immune function and increase quality of life of infected individuals are some of the goals that introduction of highly active antiretroval therapy (HAART) in HIV infection management have achieved in recent times [1]. HAART includes the combination of three different types of highly effective anti-HIV drugs including nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleotide reverse transcriptase inhibitors (NNRTIs) and non-peptidic viral protease inhibitors (PIs) [2]. The administration of these drugs have been associated with specific toxicities including mitochondrial toxicity, cardiovascular complications, liver toxicity, renal toxicity, gastrointestinal intolerance, glucose and lipid abnormalities [3]. These drug associated toxicities have resulted in greater proportion of patients developing chronic conditions not traditionally related to HIV such as cardiovascular, liver and kidney diseases [4]. The development of HAART related organ damage is a function of complex interactions between individual risk factors, HIV correlated effects and antiretroviral drug

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toxicity [3]. These long terms adverse effects often referred to as non infectious HIV related co-morbidities have become common source of morbidity and mortality among HIV infected individuals in developing countries [5].

Varying degrees of HAART related organ toxicities have been described in HIV infection. Abnormalities in renal and liver functions have been independently reported in HIV infection and HAART [6,1,7]. Studies on the nephrotoxic or hepatoxic effects of HIV infection and HAART regimen as separate entities are rife in Nigeria [1,6,7] though still relatively unexplored in Calabar, South-South Nigeria, studies on the combined nephrotoxic and hepatotoxic effects of HIV infection and HAART are however scarce and will be more effective in monitoring the dosing of HAART and the onset of adverse drug reactions. The combined liver and renal functions of HIV seropositive individuals with or without HAART were determined in this study.

Materials and Methods

Study design

This case control study which examined the combined nephrotoxic and hepatotoxic effects of HAART in HIV infection was carried out at the President's Emergency Plan For AIDS Relief (PEPFAR) clinic of the University of Calabar Teaching Hospital, Calabar, Nigeria with the approval of the ethical committee of the hospital. This study was carried out in accordance with the Ethical Principles for Medical Research involving human subjects as outlined in the Helsinki Declaration of 1975 and subsequent revisions. The study population comprised of HIV seropositive subjects on HAART, HIV seropositive subjects not on HAART (HAART Naive) and HIV seronegative subjects serving as controls.

Selection of subjects

Sixty consenting HIV confirmed sero-positive subjects (31 males and 29 females) aged 18-55 years drawn from the PEPFAR clinic were recruited as the test subjects. Thirty age and sex matched apparently healthy, HIV sero-negative volunteers drawn from staff and students of the hospital were recruited as control subjects. Subjects with full blown AIDS, pregnant women, lactating mothers and those who did not give consent were excluded from the study. The test subjects were categorized into two groups; (1) HIV seropositive subjects not on HAART (HAART naïve n= 30) and (2) HIV seropositive subjects on HAART (n = 30). The HIV seropositive subjects on HAART would have been on any of the HAART regimens corresponding with the Nigerian National guidelines for HAART [8] for a minimum of 6 months.

Socio-demographic information was obtained from all subjects of study through a semi structured questionnaire, while anthropometric indices including body weight and height were measured and body mass index (BMI) calculated.

Sample Collection

Six milliliters of whole blood samples were collected by venipuncture with minimum stasis and aliquoted as follows; 2ml was dispensed into di-potassium ethylenediaminetetre-acetic acid (K_2EDTA) tube for CD4+T cell count and 4ml into plain specimen container. The samples were allowed to clot, retract and centrifuged for 10 minutes at 3500 revolutions per minute. Serum was separated and kept immediately frozen at -20^{0}C until time of analysis.

Laboratory Methods

HIV screening and confirmation was done by immune-chromatographic methods [9]. Enumeration of CD4+ T cell count was carried out by flow cytometry [10]. Gamma-glutamyl transferase, lactate dehydrogenase, Aspartate amiotransferase and Alanine aminotransferase activities were estimated using modified method based on the recommendations of the Scandinavian Committee on enzyme [11, 12, 13, 14]. Estimation of Alkaline phosphatase activity, uric acid and creatinine levels were done by colorimetric methods respectively [15, 16, 17,] while creatinine clearance (eGFR) was calculated using Cockcroft–Gault equation [18].

Statistical Analysis

Data were analyzed using the statistical package of social sciences (SPSS version 20.0). Student's t test analysis, one way analysis of variance, LSD post hoc and spearman's correlation coefficient were used for comparison of group means, variations within and

among groups and associations between variables respectively. A probability value $p<0.05$ was considered statistically significant.

Results

The comparison of mean age, BMI, CD4+T cell count, GGT, LDH, AST, ALT, ALP, Uric acid and estimated GFR in Controls, HIV on HAART and HAART naive subjects were shown in table 1. Significant variations were observed in the BMI, CD4+T cell count, GGT, LDH, AST, ALT, ALP and Uric acid among the 3 groups studied ($p<0.05$). No significant variations was observed in the age and eGFR among the 3 groups ($p>0.05$).

Table 2 shows the comparison of BMI, CD4+T cell count, GGT, LDH, AST, ALT, ALP and Uric acid in Controls, HIV on HAART and HAART naive subjects using LSD post hoc analysis. Significantly higher BMI and CD4+T cell count and lower AST, ALT, LDH and uric acid levels were demonstrated in HIV sero-negative controls compared to and HIV HAART naïve subjects ($p<0.05$). HIV sero-negative controls also had higher BMI and CD_4 T cell count and lower GGT, LDH, AST, ALT, ALP and uric acid levels compared to HIV on HAART ($p<0.05$). HIV on HAART had higher AST, ALT, GGT and LDH activities compared to HAART naïve subjects ($p<0.05$).

Figure 1 shows correlation plot of CD4+T cell count and uric acid in HIV HAART naive subjects. A significant negative correlation was observed between CD4+T cell count and serum uric acid levels ($r = -0.881$, $p=0.000$) ($p<0.05$).

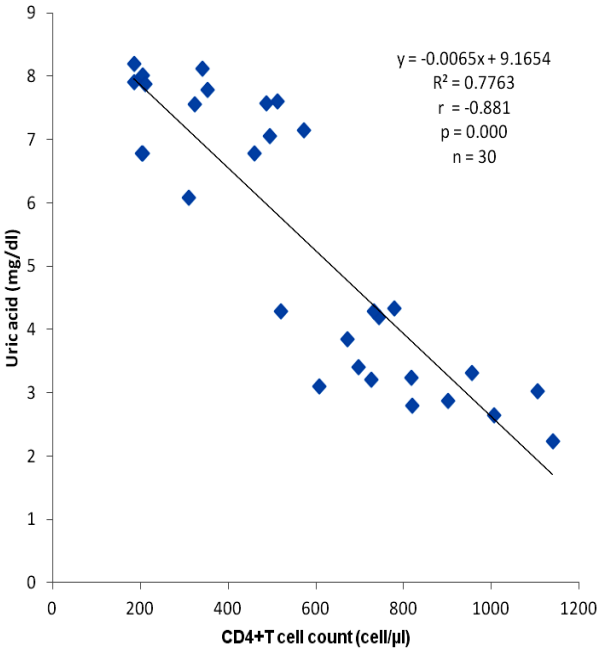

Fig.1 Correlation plot of Uric acid against CD4T cell count in HAART naïve subject

Table 1 Comparison of mean age, BMI, CD4+T cell count, GGT, LDH, AST, ALT, ALP, Uric acid and eGFR in Controls, HIV on HAART and HAART naive subjects

Index	Control n = 30	HAART Naïve n = 30	HAART n = 30	F ratio	P value
Age(years)	34.73± 8.82	32.53±8.74	35.80±8.64	1.090	.341
BMI(kg/m^2)	24.00± 2.68	21.62±2.24	22.39±1.91	8.320	.000*
CD4$^+$T(cells/ml)	1127.86±382.52	575.70±288.92	560.16±309.87	28.884	.000*
GGT(IU/L)	17.93±7.80	23.82±10.43	90.74±17.15	317.31	.000*
LDH(IU/L)	272.47±26.76	312.58±64.36	499.60±71.45	132.73	.000*
AST(IU/L)	24.63±7.23	53.03± 9.36	70.76±14.15	143.22	.000*
ALT(IU/L)	19.63±5.55	50.86±14.04	63.56±13.83	109.75	.000*
ALP(IU/L)	29.90±12.94	36.83±18.55	42.33±13.19	5.094	.008*
UA(mg/dl)	3.82±1.17	5.47±1.33	6.08±1.24	26.31	.000*
eGFR(ml/min)	98.48±26.40	75.15±16.39	84.17± 119.13	.822	.443

* = significant at $p<0.05$, BMI=body mass index, GGT=gamma glutamy transferase, LDH=lactate dehydrogenase, AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phophatase, UA=uric acid, eGFR=estimated glumerular filtration rate.

Table 2 Comparison of BMI, CD4+T cell count, GGT, LDH, AST, ALT, ALP and Uric acid in Controls, HIV on HAART and HAART naive subjects using LSD Post-hoc analysis.

Parameter	GROUPS		Mean Diff.	p value
	Controls n = 30	HAART Naïve n = 30		
BMI(kg/m^2)	24.00±2.68	21.62±2.23	2.37±.59	.000*
CD4+T(cells/ml)	1127.86± 382.52	575.70±288.92	552.16±85.09	.000*
LDH(IU/L)	272.47± 26.76	312.58±64.36	-40.10±14.88	.008*
AST(IU/L)	24.63± 7.22	53.03± 9.36	-28.40±2.75	.000*
ALT(IU/L)	19.63±5.55	50.86±14.04	-31.23±3.05	.000*
UA(mg/dl)	3.82±1.17	5.47±1.33	-1.64±0.32	.000*
	CONTROLS n = 30	HAART n = 30		
BMI(kg/m^2)	24.00±2.68	22.3933±1.91	1.61±.59	.008*
CD4(cells/ml)	1127.86±382.52	560.16±309.87	567.70±85.09	.000*
GGT(IU/L)	17.93±7.80	90.7390±17.15	-72.80±3.21	.000*
LDH(IU/L)	272.47± 26.76	499.60±71.45	-227.13±14.88	.000*
AST(IU/L)	24.63±7.22	70.76±14.15	-46.13±2.75	.000*
ALT(IU/L)	19.63± 5.55	63.56±13.83	-43.93±3.05	.000*
ALP(IU/L)	29.90±12.94	42.33±13.19	-12.43±3.09	.002*
UA(mg/dl)	3.82± 1.17	6.0827±1.24	-2.25± 0.32	.000*
	HAART n = 30	HAART NAÏVE n= 30		
GGT(IU/L)	90.7390±17.151	23.82±10.43438	66.91±3.21	.000*
LDH(IU/L)	499.60±71.4510	312.58±64.361	187.01±14.88	.000*
AST(IU/L)	70.76±14.15	53.03± 9.36421	17.73±2.75	.000*
ALT(IU/L)	63.56±13.83	50.86±14.03624	12.7±3.05	.000*

* = significant at p<0.05, BMI=body mass index, GGT=gamma glutamy transferase, LDH=lactate dehydrogenase, AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phophatase, UA=uric acid, eGFR=estimated glumerular filtration rate.

Discussion

Antiretroviral drug related organ damage are common cause of death and treatment discontinuation in HIV infection and is a major drawback to the success story of HAART in the treatment and management of HIV infection. Mechanisms for development of these disorders are still uncertain because not all of the patients exposed to the same HAART regimens are similarly affected.

In this study, higher levels of all the liver enzymes studied (ALT, AST, ALP, GGT and LDH) were observed in HIV infected subjects with or without HAART compared to HIV sero-negative controls. Elevation of the activities of liver enzymes is frequent in HIV infected patients [19]. Varying degrees have been reported with all classes of antiretroviral drugs and has been a common reason for HAART discontinuation in clinical practice. Infection of hepatocytes by HIV results in cellular damage and alteration in liver functions and release of liver enzymes depending on the severity of damage [20]. Studies have shown that structural changes in hepatocytes impair the functions of affected parts [21]. Mechanisms of HAART associated hepatotoxicity have been described including mitochondrial toxicity, hypersensitivity reactions, metabolic host mediated injury and immune reconstitution phenomena [22]. HAART can induce liver toxicity by covalent binding of drugs to cell proteins leading to disruption of cell membrane and cell death. Binding of drugs to cell proteins may also result in generation of neoantigens and induction of immunologic reactions [23]. Hypersensitivity reactions have been reported in the use of NVP and abacavir for both HIV treatment and prophylaxis after HIV exposure [22]. HAART may also induce hepatocellular damage by inhibition of mitochondrial function leading to increased generation of reactive oxygen species and lipid peroxidation, inhibition of cellular pathways of drug metabolism leading to choleostasis, jaundice and apoptosis [23]. Polymorphism of the enzyme complex responsible for drug metabolism may lead to significant heterogeneity in drug metabolism, predisposing to development of hepatotoxicity in certain individuals[22]. The estimated GFR did not vary significantly among HIV on HAART,

HIV-HAART naïve subjects and HIV sero-negative controls. This observation may be attributed to the finding that the HAART naïve subjects recruited at the time of study were newly diagnosed and as such infection may still be in its early stages and renal damage minimal while HIV on HAART subjects were those whose immune system have undergone a substantial improvement as a result of HAART, as can be seen from their $CD4^{+}$Tcell counts (575.5 versus 560.6 respectively). This could also be as a result of absence or low prevalence of traditional risk factors such as diabetes and high blood pressure within sample population which is known to be associated with renal impairment as shown by the findings of our previous study [24]. Similar observations have been made by previous studies [3, 25]. However, the eGFR of HIV sero-positve subjects on HAART were found to be higher than those of HAART naïve studied though not statistically significant. This observation may be attributed to reports that HAART in the process of reducing viral load and increasing CDT4 cell count, may also lead to a decrease in the rate of organ damage caused by HIV infection [25]. Persistent viral suppression associated with HAART has been correlated with declining incidence of chronic kidney disease and improvement in eGFR [3]. Improvement in eGFR and stabilization of renal functions has been reported after 24 months of initiation of HAART [26, 27]. Contrary to our observation, lower eGFR has been reported in individuals on HAART compared to HAART naïve subjects [3]. Mechanisms of HAART associated renal impairment may include hypersensitivity reaction, crystal formation, CytP450 interaction, mitochondrial toxicity and transporter inhibition [28].

The uric acid levels in HIV on HAART and HAART naive subjects were significantly higher compared to HIV seronegative controls. Hyperuricemia has been shown to be a common finding among individuals with HIV infection compared to the general population [29]. Hyperuricemia in HIV infection may result from complex pathological, metabolic and immunologic interactions that characterize progress of the disease from asymptomatic infection to terminal illness. HIV viremia induced increased cell turnover, loss of mononuclear cells, autoimmune or neoplastic disorders, hypercatabolic states, concomitant infections and increased oxidative stress have all been implicated in hyperuricemia in HIV infection [30, 31, 32]. Some antiretroviral drugs have been associated with mitochondrial dysfunction which may lead to increase lactate formation, which competes with urate for tubular secretion in the kidneys leading to hyperuricemia [31]. Antiretroviral drug didanosine is a purine analogue that can be degraded to urate and thus may elevate urate pools [31]. Negative correlations were observed between uric acid levels and CD4+T cell count in HIV HAART naïve subjects studied. Correlation between hyperuricemia and progression of HIV disease have been described [32, 33].

Conclusion

The findings of this study suggests that HIV infection and use of highly active antiretroviral therapy in the population studied is associated with low grade hepatotoxicity without impairment of renal functions and therefore do not warrant discontinuation of HAART. However, routine estimation of renal and liver functions of these individuals are important surveillance tools for monitoring drug related organ toxicities.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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