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Improved Manganese Status and Increased Selenium Deficiency Following HAART Combination Therapies in Nigerians with HIV-1 Infection

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Abstract: To determine the influence of two different Highly Active Antiretroviral Therapy combinations (HAART) on lipid peroxidation and some selected trace metals. The 120 HIV-1 positive patients were on two different Highly Active Antiretroviral Therapies (HAART) for at least 6 months; 60 patients were on Zidovudine + Lamivudine + Nevirapine (HAART-I) and another 60 were on Zidovudine + Lamivudine + Efavirenz (HAART-II). The 60 newly diagnosed HIV-1 positive patients who were on no treatment (pre-HAART) and 64 non-HIV infected individuals were recruited as controls. Malondialdehyde was measured using commercial kits based on modified, rapid, sensitive and specific Thiobarbituric Acid (TBARS) Method. Serum Manganese (Mn), Selenium (Se), Zinc (Zn) and Copper (Cu) were determined by flame atomic absorption spectrophotometry. Plasma MDA level was significantly higher ($p<0.05$) in HIV-1 patients on HAART-II compared to HAART-I, 0.37±0.10 vs. 0.28±0.19 μmol L^{-1}. HAART-I and HAART-II combinations brought about a significant increase in serum $p<0.05$, respectively. Conversely, a significant reduction in serum levels of Se were observed in both HAART-I and HAART-II subjects compared with pre-HAART, 18.02±2.89, 17.36±2.21 vs. 30.01±5.18 μg dL^{-1}, $p<0.05$, respectively. A significant elevation in serum Zn level was observed in HAART-I group while a significant decrease was observed in HAART-II group compared with pre-HAART group, 87.00±4.39, 72.91±12.48 vs. 76.75±10.01 μg dL^{-1}, $p<0.05$, respectively. Significant reduction were observed in serum levels of Cu in both HAART-I and II groups compared with pre-HAART, 64.66±5.00, 68.66±4.63 vs. 69.26±5.91 μg dL^{-1}, $p<0.05$, respectively. The final effect of HAART related oxidative imbalance in HIV infected individuals depends to some extent on the HAART combination and the interplay between the degree of selenium deficiency and manganese restoration.

Key words: HAART toxicity, trace elements, malondialdehyde, HIV, TBARS

INTRODUCTION

With the advent of Highly Active Antiretroviral Therapy (HAART), the management of HIV/AIDS has taken a dramatic turn however, the side-effects of HAART in HIV management cannot be overlooked (Ankikar *et al*., 2011; Li *et al*., 2011).

In recent time, the use of HAART has been associated with some serious side effects which includes increased free radical generation and its attendant free radical injury via Reactive Oxygen Species (ROS) (Shahar *et al*., 2008; Sundaram *et al*., 2008), hepatotoxicity (Jones and Nunez, 2012; Lopez-Delgado *et al*., 2012), cardiotoxicity (Casaretti *et al*., 2012; Lipshultz *et al*., 2012), dyslipidemia and lipodystrophy (Domingo *et al*., 2012), renal toxicity (Calza, 2012) and a host of others. The presence of HIV/use of HAART has also been implicated in the alteration of metabolism of some essential micronutrients such as Selenium (Se), Zinc (Zn), Manganese (Mn) and Copper (Cu) however, findings in

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this regard varies from one centre to another (Abdollahi and Shoar, 2012; Akinola *et al*., 2012, Bunupuradah *et al*., 2012; Okwara *et al*., 2012). There is therefore a need for a re-evaluation of the effect of the presence of HIV/use of HAART on free radical generation and micronutrient homeostatic disturbances in Nigerians with HIV-infection.

Researchers therefore set out to determine the effect of two combination therapies commonly used in the centre; Zidovudine + Lamivudine + Nevirapine (HAART-I) and Zidovudine + Lamivudine + Efavirenz (HAART-II) onfree radical level (using serum Malondialdehyde (MDA) as indicator) and also determine their effects on serum manganese, selenium, copper and zinc homeostasis. This researchers hope to achieve by measuring the serum levels of Malondialdehyde (MDA), selenium, zinc, copper and manganese. It is hoped that results obtained will be a pointer to the degree of free radical generation and trace metal homeostatic disturbances in each HAART combination.

MATERIALS AND METHODS

The setting for this study was the Institute of Human Virology of Nigeria (IHVN) centre located inside Ladoke Akintola University of Technology Teaching Hospital (LAUTECH) complex. This is a cross-sectional study of 180 HIV-1 infected Subjects attending the HIV out-patient clinic at LAUTECH Teaching Hospital. They were recruited into the study after obtaining approval from the Ethical Committee of the hospital and each subject's informed consent. Each consenting subject was successively distributed into 3 group base on medication status until a total of sixty were achieved in each group. The first group comprise 60 newly diagnosed HIV-1 subjects who are not on any antiretroviral medication (pre-HAART), the second group comprise another 60 HIV-1 infected subjects who had been on HAART-I combination (Zidovudine + Lamivudine + Nevirapine) for at least 6 months while the third group comprise 60 HIV-1 infected subjects who had been on HAART-II (Zidovudine + Lamivudine + Efavirenz) for at least 6 months. A total number of 64 (apparently healthy) consenting age and sex matched HIV-1 and 2 negative, Hepatitis B and C negative volunteers were recruited from those who had come for voluntary HIV screening and were found negative.

After an overnight fast, 10 mL^{-1} of blood was drawn from the antecubital vein of each subject and control using appropriate techniques. The 3 mL were dispensed into heparin EDTA bottles for CD4+ count within 6 h of sample collection and the rest 7 mL dispensed into plain acid washed containers for trace metals and malondialdehyde estimation. Samples in the plain bottles were allowed to clot and retract, centrifuged at 3,000 rpm for 5 min and serum harvested into another set of acid washed glass tubes and later stored at -20°C prior to analysis. Analysis for each of the 3 groups of HIV-1 subjects and controls were carried out in the same batch. Malondialdehyde was measured using commercial kits based on modified, rapid, sensitive and specific Thiobarbituric Acid (TBARS) Method (Botsoglou *et al*., 1994). Serum concentrations of trace metals (Se, Zn, Cu and Mn) were estimated by Flame Atomic Absorption Spectrophotometric Method with the aid of Flame Atomic Absorption Spectrophotometer serial number SN 115863752DJ and plasma CD4+ count was determined by flow cytometric method using Scan Flow Cytometry (Beckton-Dickinson, Rutherford, New Jersey, USA).

Data obtained were subjected to statistical analysis with the aid of Statistical Package for Social Sciences (SPSS) Version 18.0 Software package and results are expres sed as mean and standard deviation when compared using the independent t-test regarding $p \leq 0.05$ as significant.

RESULTS AND DISCUSSION

The age and sex distribution of study is as displayed in Table 1. As shown in Table 2, there were no statistical differences in mean age for both subjects and controls. Mean CD4+ count were significantly higher in controls compared with pre-HAART, HAART-I and HAART-II subjects, 1094.83±278.86 vs. 505.53±389.73 lymp/mm^3, 699.63±539.85 and 471.37±276.60 lymp/mm^3, $p<0.05$, respectively. A significant reduction in mean CD4+ count was observed in subjects on HAART-II compared with pre-HAART and HAART-I groups ($p<0.05$, respectively) (Table 3). The mean MDA levels in controls were not significantly different from levels obtained in pre-HAART and HAART-I subjects however subjects on HAART-II demonstrates a significantly higher MDA levels compared with controls and subjects on HAART-I, 0.37±0.10 vs. 0.19±0.13 and 0.28±0.36 μmol L^{-1}, $p<0.05$, respectively. Manganese level in controls although, slightly higher are not significantly different from those on HAART-I and

Table 1: Age and sex distribution of study population

	Pre-HAART (N = 60)		HAART-I (N = 60)		HAART-II (N = 60)		Control (N = 64)	
Age (years)	Male	Female	Male	Female	Male	Female	Male	Female
21-30	4	8	6	14	16	10	18	16
31-40	8	14	12	14	12	10	8	6
41-50	8	12	2	6	6	2	8	4
>50	2	4	4	2	4	-	4	-

Table 2: Comparison of Mean±2SD of variables in the study population control vs. pre-HAART, HAART-I and HAART-II

Variables	Pre-HAART (n = 60)	HAART-I (n = 60)	HAART-II (n = 60)	Control (n = 64)
Age (years)	38.73±9.0000	35.07±9.4900	34.43±8.4500	34.90±13.680
CD4+ (lymp/mm^3)	505.53±389.73*	699.63±539.85*	471.37±276.60*	1094.83±278.86
MDA (μmol L^{-1})	0.22±0.2600	0.28±0.3600	0.37±0.1000*	0.19±0.1300
Mn (μg dL^{-1})	56.38±14.470*	86.58±7.5300	98.15±17.430	101.38±34.820
Cu (μg dL^{-1})	69.26±5.9100*	64.66±8.0000*	68.66±8.0000*	81.90±17.870
Zn (μg dL^{-1})	76.75±10.010	87.00±4.3900*	72.91±12.480*	67.41±4.9100
Se (μg dL^{-1})	30.01±5.1800*	18.02±2.8900*	17.36±2.2100*	32.79±5.4600

*Significant difference in mean compared with controls at p<0.05

Table 3: Pre-HAART vs. HAART-I and HAART-II

Variables	Pre-HAART (n = 60)	HAART-I (n = 60)	HAART-II (n = 60)
Age (years)	38.73±9.0000	35.07±9.4900	34.43±8.450
CD4$^+$ (lymp/mm^3)	505.53±389.73	699.63±539.85*	471.37±276.6
MDA (μmol L^{-1})	0.22±0.2600	0.28±0.3600	0.37±0.100*
Mn (μg dL^{-1})	56.38±14.470	86.58±7.5300*	98.15±17.43*
Cu (μg dL^{-1})	69.26±5.9100	64.66±8.0000*	68.66±8.000*
Zn (μg dL^{-1})	76.75±10.010	87.00±4.3900*	72.91±12.48*
Se (μg dL^{-1})	30.01±5.1800	18.02±2.8900*	17.36±2.210*

*Significant difference in mean compared with Pre-HAART at p<0.05. Mean±2SD represents the ±values

Table 4: HAART-I vs. HAART-II

Variables	HAART-1 (n = 60)	HAART-II (n = 60)	p-value
CD4+	699.63±539.85	471.37±276.6	p<0.05
MDA	0.28±0.3600	0.37±0.100	p<0.05
Mn	86.58±7.5300	98.15±17.43	p<0.05
Cu	64.66±8.0000	68.66±8.000	p<0.05
Zn	87.00±4.3900	72.91±12.48	p<0.05
Se	18.02±2.8900	17.36±2.210	p>0.05

Mean±2SD represents the ±values

HAART-II (101.38±34.82 vs. 86.58±7.53 and 98.15±17.43 μg dL^{-1}, p>0.05, respectively) (Table 4), however, manganese level in pre-HAART subjects were significantly reduced compared with controls, HAART-I and HAART-II subjects; 56.38±14.47 vs. 101.38±34.82, 86.58±7.53 and 98.15±17.43 μg dL^{-1}, p<0.05, respectively. Copper levels are significantly higher in controls than in pre-HAART, HAART-I and HAART-II subjects, 81.90±17.87 vs. 69.26±5.91, 64.66±8.00 and 68.66±8.00 μg dL^{-1}, p<0.05, respectively.

Similar levels of zinc were found in controls and PREHAART subjects, however, HAART-I and HAART-II subjects demonstrates a statistically significant higher values compared with controls and pre-HAART subjects, 87.00±4.39 and 72.91±12.48, vs. 67.41±4.91 and 76.75±10.01 μg dL^{-1}, p<0.05, respectively. Selenium levels in controls were significantly higher compared with pre-HAART, HAART-I and HAART-II subjects, (32.79±5.46 vs. 30.01±5.18, 18.02±2.89 and 17.36±2.21 μg dL^{-1}, p<0.05, respectively. A significantly reduced Selenium level was observed in both HAART-I and HAART-II groups compared with pre-HAART status however, no significant difference was observed between serum Selenium levels in HAART-I and HAART-II subjects.

The use of HAART has been associated with disturbances in membrane lipid peroxidation and it's accompanied alteration in the body's ability to maintain oxidative balance moreover, alterations in some key trace metals with powerful antioxidative property have been reported (Abdollahi and Shoar, 2012; Akinola *et al*., 2012; Bunupuradah *et al*., 2012; Okwara *et al*., 2012). These reported effects both on free radical generation and trace metal homeostasis varies from one place to another. Micronutrient deficiencies are common among HIV-1 infected persons who are underprivileged and malnourished (Semba and Tang, 1999) and this may exacerbate immunosupression, oxidative stress, acceleration of HIV replication and CD4+ T-cell depletion (Bodgen and Oleske, 2007).

Apart from environmental factors, the type of combination in each HAART composition may have a role to play in these observed variations this study was therefore designed to determine the influence of two common HAART combination (HAART-I, Zidovudine + Lamivudine + Nevirapine and HAART-II, Zidovudine + Lamivudine + Efavirenz) therapies used in our centre on free radical levels using Malondialdehyde (MDA) level as indicator and also determine their effects on serum zinc, selenium, copper and manganese homeostasis.

Malondialdehyde (MDA): Prior to the commencement of HAART, the MDA levels were slightly higher in pre-HAART subjects than those found in HIV negative controls however, this was not statistically significant, the introduction of HAART-I and HAART-II brought about a further increase in MDA levels with a statistically significant increase in subjects on HAART-II only. This implies that the presence of HIV infection in the subjects is not associated with significant increases in free radical generation but the commencement of HAART, especially, HAART-II. This findings are similar to an earlier research from Cameroon that concludes HIV infection increases oxidative stress process which is further compounded by the use of HAART (Ngondi *et al*., 2006). However, the findings are similar for HAART-I but contrary in HAART-II subjects to reports from South India and Israel that reported no significant difference in oxidative status in HAART experienced subjects and a return to normal of oxidative profile following HAART therapies, respectively

(Shahar *et al*., 2008; Sundaram *et al*., 2008). Subjects on HAART-II combination can be said to be more exposed to HAART induced toxicity than those on HAART-I combination as indicated by significant increase in MDA levels in this group of subjects. Since, HAART-I and HAART-II differs only in the substitution of Nevirapine in HAART-I for Efavirenz in HAART-II, it is tempting to infer that Efavirenz is a more toxic component when included in HAART combination therapy. The observation on Efavirenz is in support of earlier studies where Efavirenz based combinations and high plasma Efavirenz has been shown to increase hepatocellular damage (Bumpus, 2011; Yimer *et al*., 2011; Mugusi *et al*., 2012).

Selenium: The association between HIV infection and selenium deficiency is well documented however, the effect of HAART on serum levels of selenium in the management of HIV remains controversial. Various findings have been reported with regards to the effect of HAART on Selenium status. Some reports have associated the use of HAART with a reduction in Selenium levels (Shor-Posner *et al*., 2002; Atiba *et al*., 2012) while others have actually reported an increase (Rousseau *et al*., 2000) and no association in some cases (Jones *et al*., 2006; Akinola *et al*., 2012; Bunupuradah *et al*., 2012). In this present study, selenium levels prior to commencement of HAART (pre-HAART) were significantly reduced compared with HIV-negative controls, the introduction of HAART in the form of HAART-I and HAART-II combinations brought about a further drastic reduction in serum selenium levels. The pattern of reduction in serum selenium levels in both HAART-I and HAART-II combinations are similar both brought about almost a fifty percent reduction in serum selenium levels in the subjects. The present study is in agreement with the findings that there is some degree of selenium deficiency associated with HIV infection and that the introduction of HAART further complicates selenium deficiency in HIV infected persons receiving HAART treatment. Selenium through its selenoproteins is well known for its powerful antioxidative property therefore, it's deficiency in HIV infected persons and the attendant further drastic reduction through the introduction of HAART may further jeopardize the total antioxidative capacity in individuals with HIV on HAART therapy. Selenium supplementation has been advocated in the management of HIV however, this has been advised to be carefully moderated in terms of associated risks and benefits (Pitney *et al*., 2009).

Manganese: There is a dearth of literatures regarding the influence of HAART on serum manganese levels. In this study, manganese levels were significantly reduced in HIV subjects who are yet to commence HAART combination therapy compared with HIV-negative controls however, a significant improvement in manganese level was brought about by the commencement of HAART-I and HAART-II, respectively. The overall picture seems to suggest that there is a reduction in the serum manganese level associated with HIV infection which improves significantly by the institution of HAART. Manganese is an integral component of the powerful antioxidant enzyme Superoxide-Dismutase (MnSOD) through this enzyme; manganese has been recognized as a powerful antioxidant trace element. The significant increase in serum manganese levels brought about by the institution of HAART is therefore a welcomed relief with regards to boosting the total antioxidative capacity in individuals with HIV infection on HAART therapy. More importantly, the significant reduction in serum selenium and the anticipated reduction in the total antioxidative capacity in the body are being neutralized to some extent by the accompanied increase in serum manganese level. Therefore, the final effect of HAART related oxidative imbalance in HIV infected individuals depends to some extent on the interplay between the degree of selenium deficiency and manganese restoration. Although, not earlier reported, symptoms suggestive of manganism (a condition associated with excess manganese in the body usually secondary to chronic manganese exposure, similar in presentation to Parkinson's disease) should be watched out for in HIV patients on HAART.

Copper: Copper has been shown to inhibit the protease from HIV-1 by both cysteine-dependent and Cysteine-independent mechanisms (Karlstrom and Levine, 1991), however, the effect of HAART treatment on copper metabolism is still unclear. In this study, copper levels are significantly reduced in pre-HAART subjects compared with HIV-negative controls. Therefore, the presence of HIV seems to be associated with significant decrease in copper levels. The institution of HAART in form of HAART-I and HAART-II further brought about a slight but significant decrease in the serum levels of copper. However, this finding is not in agreement with a earlier study from this region that documents no significant difference in mean serum selenium and copper levels between pre-HAART and subjects on HAART (Akinola *et al*., 2012). From the study on the effect of HAART on serum copper levels in HIV infected

individuals; it is highly suggestive that the institution of HAART further reduces copper levels in HIV subjects.

Zinc: The trace element zinc is involved in many important immune processes however, observational epidemiologic studies have provided conflicting results on the role of zinc status in HIV disease progression (Kupka and Fawzi, 2002). Zinc levels in the pre-HAART subjects were not significantly different from those in HIV-negative controls suggesting that HIV infection is not significantly associated with altered serum zinc levels. However, commencement of HAART brought about different pattern of alterations in subjects on HAART-I and those on HAART-II. HAART therapy brought about a significant increase in Zn levels in subjects on HAART-I while a reversed case of significant reduction in serum Zn levels were found in subjects on HAART-II. Therefore, the effect of HAART on serum zinc homeostasis is combination dependent.

CONCLUSION

The final effect of HAART related oxidative imbalance in HIV infected individuals depends to some extent on the HAART combination and the interplay between the degree of selenium deficiency and manganese restoration.

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