Effect of HIV and HAART on Antioxidants Markers in HIV Positive Patients in Sokoto State, Nigeria

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Abstract- The study assessed the effect of both HIV and highly active antiretroviral therapy (HAART) on antioxidant markers in patients with HIV infection being managed with HAART. Fifty (50) HIV positive HAART naïve (12 male and 38 females), fifty (50) HIV positive on HAART for 1 to 6 months (18 male and 32 females), fifty (50) HIV positive on HAART for 7 to 12 months (14 males and 36 females) and fifty (50) HIV negative (28 males and 22 females) were enrolled into the study. Oxidative stress markers such as reduced glutathione (GSH), Malondialdehyde (MDA), Catalase, Vitamin A, C and E were measured using different standard methods. Significantly ($p<0.05$) low levels of reduced glutathione in non-treated group were observed compared to the control. The three HIV positive groups have significantly higher MDA and lower Catalase activity when compared to control. Vitamins A, C and E show a significant decrease ($p<0.05$) in all the HIV positive patients when compared with control. HIV infection increases oxidative stress, which decreases at the commencement of therapy (HAART). Antioxidants supplementations should be considered for incorporation in the management of HIV/AIDS patients.

Key words: catalase, malondialdehyde, reduced glutathione, vitamin A, C, E.

1. INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a serious infection of the human immune system caused by a retrovirus known as Human Immunodeficiency Virus [1]. The HIV is known to infect major components of the human immune system such as CD_4 T-cells, macrophages and dendritic cells, directly or indirectly destroying CD_4 T-cells [2].

A report released by UNAID in 2013[3] showed that as of 2012 nearly 35.5 million people were living with HIV worldwide, with the number of new infection being about 2.3 million. The report also indicated that approximately 16.8 million are women. In 2012 it was estimated that the disease claimed approximately 1.6 million lives, which was down from the peak of 2.2 million in 2005 [4].

In Sub-Saharan Africa, which is the most affected region the prevalence figure released in 2010 indicated that an estimated 68% (22.9 million) people are living with the disease and 66% of all the global deaths (1.2 million) occurred in the region [4].

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In Nigeria, a 2012 statistics showed that, the number of people living with HIV was 3.4 million with adult prevalence rate of 3.1% [5]. UNAID[5] also reported that worldwide, Nigeria has the second highest number of new infection reported each year with an estimated of 3.7% of the population are living with HIV and also in 2011 alone, about 210,000 people died due to AIDS.

Human Immunodeficiency Virus (HIV) induces chronic immune activation mediated by increased generation of reactive oxygen species (ROS) and overwhelming the antioxidants defense system [6]. Oxidant stress has been observed in many HIV infected individuals [7].

In 2010 all estimated 6.6 million people were taking antiretroviral therapy in developed and undeveloped countries [4], which is the main strategy for management of the disease.

Highly active antiretroviral therapy (HAART) has greatly extended survival, with reduction of the morbidity and mortality of HIV infection, though the drugs cannot totally eliminate the virus [8]. Edward*et al.*[8] HAART does not completely solve the immune and metabolic alteration during HIV infection.

Some antiretroviral drugs have been associated with increased oxidative stress in people living with HIV/AIDS [9]. HAART induces oxidative stress (OS) [10; 11].

Herzenberg *et al.* [12] reported a significant decrease in levels of reduced glutathione, which has been associated with impaired T-cell function and survival in HIV infection. Decrease in the activities of some antioxidants enzymes like Catalase in HIV positive subjects at base line has been reported

[13]. High level of Malondialdehyde has been reported in both symptomatic and asymptomatic HIV infected patients [14].

Vitamin A is an essential component of the immune system that regulates white bloodcell production to prevent, thwart off, and destroy bacteria and viruses that cause infection [15]. Vitamin A is essential in immune response of macrophages, which areone of the first cells at the onset of HIV-infection, it maysignificantly, directly or indirectly, be responsible for thedepletion of a large number of CD_4^+ T-cells and disease progression [16]. Eylar*et al.*[17] reported that both vitamin C dosage and duration are essential in suppressing HIVinfection. Vitamin E deficiencies and oxidative stress have been associated with HIV-seropositive patients [18; 19]. A study of 296 HIV-infected men showed a decrease in the risk of progression of AIDS by doubling their vitamin E intake [20].

The study aimed to assess the effect of both HIV and HAART on oxidative stress indexes, to also determine the pattern of abnormalities at different period of antiretroviral treatment and the potential risk it poses (HAART) to patients who are about to enrol on the treatment in the state.

2. Materials and Methods

The research was carried out in Sokoto State, Nigeria. Fifty (50) HIV positive patients HAART naïve (pre-HAART subjects), Fifty (50) being on HAART for 1 to 6 months and Fifty (50) for 7 to 12 months on HAART were recruited into the study. The control groups were fifty (50) randomly selected healthy individuals who were tested HIV negative. The ethics and research committee's clearance of Usmanu Danfodiyo University Teaching Hospital, Sokoto and Specialist Hospital, Sokoto were obtained and all subjects gave informed consent before participating in the study. Exclusion criteria for the study includes: Smoking, Diabetes, Kidney disease, Liver disease, and diarrhea (more than six liquid stools per day).

2.1 Sample Collection and Treatment

Twelve (12) hours fasting venous blood (5ml) was collected from all subjects using multi sample needle with sterile vacutainer specimen bottles. The specimens were centrifuged at 3000g for 5 minutes. The serum was removed and transferred into serum containers for the analyses.

2.2 Method of assessment

Reduced glutathione (GSH) was determined by the method of Patterson and Lazarow [21]. Catalase activity was assessed based on enzymatic method of Beers and Sizer [22]. Malondialdehyde (MDA) was determined by method of Shah and Walker's [23]. Vitamins A, C and E were assayed using methods of Bassey *et al.* [24], Tietz [25], Baker and Frank [26] respectively.

2.3 **Reagents:** All reagents used in the study were of analytical grade.

3. Statistical Analysis

The statistical analysis was performed using Graph Pad Instant version 3.05, 32 bit for win 95/NT. Values are expressed as Mean ± Standard error of mean. Benferroni compare all columns was used to assess the differences ($P<0.05$) between the groups. A P value less than 0.05 was taken as an indication of significant difference.

4. Results

HIV positive patients have significantly lower levels of reduced glutathione (GSH) compared to the control group. Patients on HAART for 7-12 months have higher levels of GSH compared to others. Significantly ($P< 0.05$) lower level of glutathione in female non-treated group compared to female control subjects (Table 2) was observed. Malondialdehyde levels are significantly higher in patients with HIV compared to the control (Table 1). A significant decrease in the level of MDA of female control was observed when compared to non-treated and subjects treated for 1 to 6 months (Table 2). Catalase activity was significantly ($p<0.05$) lower in the treatment naive compared to the control (Table 1). Serum vitamin A concentration was significantly ($p<0.05$) lower in the HIV positive patients treatment naive group compared to control. Patients on ART treatment for 1 to 6 months have significantly higher levels treatment naive group. Likewise, the vitamin A levels were significantly higher in subjects on HAART for 7 to 12 months compared to those on treatment for 1-6 months (Table 1). Furthermore, significantly lower level of vitamin A in HIV positive male and female treatment naïve, those on HAART for 1 to 6 months and 7-12 months were observed compared to the control group (Table 2).

The treatment naive (non-treated) patients, patients on treatment (1 to 6 months and 7 to 12 months) had lower concentration of vitamin C compared to HIV negative subjects (control) as shown in table 1. Male control group were also observed to have significantly higher level of vitamin C compared to HIV infected males non-treated with HAART (Table 2). Levels of vitamin E of patients not on HAART, and patients who are 7 to 12 months on HAART were significantly lower ($P<0.05$) compared to control group (Table 1). Significantly higher level of vitamin E was also noticed in female control compared to HIV infected females not on HAART (Table 2).

Table 1: Effect of HIV and HAART on Antioxidants Indices of the Study Subjects in Sokoto

Antioxidants	Control	HAART naive	1-6 Months	7-12 Months
GSH (mg/dl)	45.6±2.3[a]	30.5±2.5[a,b]	36.9±2.3	42.1±2.7[b]
MDA (nmol/L)	210.5±5.4[a,b,c]	352.3±28.7[a]	347.9±10.0[b]	295.0±21.2[c]
Catalase (IU/L)	61.6±2.9[a,b]	44.7±3.3[a]	48.4±2.9[b]	52.1±3.7
Vitamin A (µmol/L)	2.3±0.1[a,b,c]	1.3±0.1[a,d,e]	1.7±0.1[b,d,f]	2.0±0.1[c,e,f]
Vitamin C (µmol/L)	76.6±2.1[a,b,c]	52.1±4.0[a]	56.7±3.9[b]	62.7±3.5[c]
Vitamin E (µmol/L)	21.4±0.4[a]	18.1±0.7[a,b]	19.5±0.5	20.8±0.4[b]

KEY: n= sample size (50/group) except: GSH (n=45), MDA (n=45), Catalase (n=44), Vitamin A (n=44), Vitamin C (n=47) and Vitamin E (n=45) of non-treated subjects. GSH (n=49), MDA (n=49), Vitamin E (n=48) of those on treatment for 1 – 6 months. Vitamin E (n=48) of treated subjects for 7 – 12 months. Values are expressed as Mean ± SEM. Values with the same superscript in the same row are significantly different ($P < 0.05$)

Table 2: Effect of HIV and HAART on Antioxidants Indices by Gender of the Study Subjects in Sokoto

Antioxidants	Control M(n)=28, F(n)=22	Treatment naive M(n)=12, F(n)=38	1-6 Months M(n)=18, F(n)=32	7-12 Months F(n)=36, M(n)=14
GSH (mg/dl)				
- Male	45.5±2.8	34.7±3.9	35.2±2.9	44.9±4.2
- Female	45.8±4.0[a]	28.9±3.1[a]	37.9±3.2	40.9±3.3
MDA (nmol/L)				
- Male	206.7±7.0[c]	284.9±57.1	362.5±15.8[c]	295.3±38.9
- Female	215.3±8.4[a,b]	376.9±32.7[a]	339.4±12.9[b]	294.9±25.6
Catalase (IU/L)				
- Male	66.4±4.5[a]	47.5±7.6	44.4±4.9[a]	57.4±6.4
- Female	55.6±3.0	43.7±3.6	50.7±3.7	49.9±4.5
Vitamin A (µmol/L)				
- Male	2.3±0.060[a,b]	1.4±0.2[a,c]	1.8±0.1[b]	2.1±0.1[c]
- Female	2.3±3.1[d,e]	1.3±0.1[d,f]	1.6±0.1[e]	1.9±0.1[f]
Vitamin C (µmol/L)				
- Male	78.3±3.1[a]	49.8±6.3[a]	57.1±6.2	61.5±6.8
- Female	74.4±2.7	53.5±4.9	56.4±5.2	63.2±4.1
Vitamin E (µmol/L)				
- Male	21.3±0.5	18.2±1.3	18.9±0.8	20.2±1.5
- Female	21.4±0.6[a]	18.1±0.8[a,b]	19.8±0.7	21.2±0.5[b]

KEY: Values are expressed as Mean ± SEM
Values with the same superscript in the row are statistically significant ($P<0.05$)
n= Sample size, M=Male, F=Female.

5. Discussion

Studies [15, 27, 28, 29] reported that HIV infection is associated with oxidative stress caused by reactive oxygen species which promotes the progression of HIV to AIDS. Further studies by Derenz [27] and Wang [30] reported that antiretroviral drugs (ARVs) increase the oxidative stress. These two factors lead to unhealthy situation in people living with HIV/AIDS which can be further aggravated by factors like diarrhea, loss of appetite, poor absorption of nutrients and low dietary intake which are all associated to both HIV and ARVs [31]. This study therefore set out to examine whether some factors (viral virulence, genetic, race and environmental difference) modify the outcome of management of AIDS patients and whether some measures need to be taken to ensure that better therapies (regimen) are received by the people living with HIV in the state.

Remarkable depletion in the level of glutathione in the HIV positive non-treated with antiretroviral drugs (ARVs) group compared to control subject may be due to being cysteine one of precursors of glutathione and HIV infection is associated

with massive catabolism of cysteine into sulfate, which can be detected at early asymptomatic stage of disease through urinary sulfate excretion as reported by Breitkreutz*et al.* [32]. The study by Droge and Breitkreutz [33] further revealed that excessive cysteine catabolism proceeds largely at expenses of glutathione rather than protein but the mechanism is unclear. HIV infection induces intracellular depletion of glutathione level so as to facilitate the induction of signaling pathway leading to lymphocyte activation, and render the cells more sensitive to oxidative stress [34].

The result of this study was is in line with that of Stephensen *et al.* [35] and Herzenberg *et al.* [12]who reported significantly lower level of reduced glutathione in HIV positive patients compared to control. HAART produces maximal suppression of viral replication to prevent disease progression, preserve immunological function and reduce opportunistic infections [36]. The massive catabolism of cysteine which subsequently leads to extensive depletion of glutathione level due to HIV infection as stated may be a reason for decreased level of glutathione in HIV positive non-treated group compared to the control group, but at the initiation of antiretroviral therapy which suppresses the HIV replication and subsequent infection, the level of glutathione increases as observed in the two treated groups (1 to 6 months and 7 to 12 months) compared to non-treated group as observed.

The findings in this study are consistent with a study conducted by others [37, 38, 39, 40, 41] who reported increase in MDA level of HIV positive patients (pre-HAART) and on HAART when compared with control subject. High level of MDA observe in this study may be due to unavailability of micronutrients, immune system degradation which generate oxyradicals, inflammation and releases cytokines to initiate neutrophils and macrophages to produce free radicals which subsequently enhances lipid peroxidation often lead to oxidative stress and cell apoptosis [42,43]. The free radicals produced as a result of HIV infection are accompanied to weight loss, decrease immune cells, extensive loss of immune function which subsequently results in massive peroxidation of polyunsaturated lipid [44]. The significantly higher level of MDA in the HIV positive female non-treated group compared to the control group may be due to increase in the activities of lipoxygenase which is mediated by cytokine levels [45]. Different cells of the immune system such as neutrophils and leucocytes contained lipoxygenase enzymes which are the principal enzymes catabolism of phospholipid from cell membrane [46]. High level of MDA in HIV positive female non-treated compared to control group may be due to the higher stored phospholipid in females and high release of neutrophil and leucocytes due to HIV infection.

Several reports [13, 35, 47, 48] show lower activities of catalase in HIV positive non-treated and those treated with HAART when compared to control subject. The decrease in the activities of catalase in the non-treated group may be due to increase utilization of endogenous enzymes like catalase and superoxide dismutase (SOD) as a result of increase in reactive oxygen species by HIV infection. Following the initiation of antiretroviral therapy the activities of catalase increases which show progressive treatment and clearance of reactive oxygen species by ARVs.

Low levels of vitamin A, C and E observed may be as a result of excessive need to overcome the immune abnormalities, detoxification of free radicals and inhibition of HIV. Symptoms of the disease (HIV/AIDS) and side effect of ARDs such as diarrhea, loss of appetite, low dietary intake and mal-absorption may leads to decrease in the levels of the vitamins. Lipid helps in absorption of fat soluble vitamins (A and E), it acts as a solvent for the transport of fat soluble vitamins [46]. Lipid peroxidation observed in the study may also be a reason for the deficiency of the vitamins (A and E) observed [46].

The result of our study is consistent with previous studies which observed significant decrease in vitamin A, C and E levels in HIV positive patients with and without antiretroviral therapy compared to control [41, 49, 50].

6. Conclusion

Decrease in antioxidant indices were experienced among the HIV positive patients with and without HAART compared to HIV negative subjects. Therefore antioxidants and micronutrients supplementation should be considered in the management of AIDS patients to overcome the oxidative stress. The study is limited to 12 month duration of therapy, which may be short in assessing the OS caused by HAART. Dietary information and vitamin A, C and E dietary intakes of the patients are not collected, therefore no comment on its role.

7. Conflict of Interest

The authors have declared no conflict of interest. Authors have not received any financial support from any organization. The authors further declare that no other relationships or activities that could appear to have influenced this study.

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