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Imbalance of antioxidant enzymes activities and trace elements levels in Ghanaian HIVinfected patients

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Abstract

Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART) have been associated with high oxidative stress in HIV patients. The disparity in antioxidant-oxidant levels in HIV patients favours viral replication and disease progression. This study aimed at determining the effect of ART on antioxidant enzymes activities and trace elements levels in Ghanaian HIV patients. A total of 242 participants; comprising of 105 HIV-infected patients on ART, 77 HIV-infected ART-naïve, and 60 HIV seronegative controls were recruited for the study. Whole blood was collected and used for haematological profiling, and the determination of CD4⁺ counts, superoxide dismutase (SOD) activity and trace element levels. Serum was used for liver function tests and the determination of glutathione reductase (GR) activity, and plasma was used to estimate reduced glutathione (GSH) levels. Low levels of haemoglobin (HB), hematocrit, mean cell volume (MCV) and mean cell hemoglobin (MCH), and trace elements were found in ART-naïve patients compared to those on ART and the seronegative controls. In the ART-naïve patients, glutathione reductase (GR) activity and reduced glutathione (GSH) level were significantly low compared to patients on ART and seronegative controls. Activity of SOD was significantly reduced in ART-naïve patients compared to those on ART and the control group, and manganese is the only trace element that showed a strong negative correlation with SOD activity and a positive and significant correlation with CD4⁺ count, and therefore needs to be investigated further. The study suggests that assessing antioxidant levels or enzymes activities of patients infected with HIV should be considered during therapy.

Introduction

Human immunodeficiency virus (HIV) infection and/or highly active antiretroviral therapy (HAART) tilts antioxidant-oxidant balance of the host towards the oxidants, with consequential poor disease outcomes [1,2]. Elevated levels of oxidative stress markers were reported in HIV-infected patients [3,4], and the levels of the markers were associated with low CD4⁺

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count and increased viral load [5]. Increased levels of reactive oxygen species (ROS) reported in HIV-infected individuals promoted nucleic acid and lipid oxidations, resulting in elevated levels of 8-Oxoguanine (8-oxoG) and malondialdehyde (MDA), respectively, as evidences [3,6]. The elevated levels of the oxidative stress-induced metabolites have also been shown to independently predict disease progression and mortality in HIV patients [7].

Capacity to clear reactive oxygen species (ROS), either in symptomatic or asymptomatic HIV patients, largely depends on the bioavailability of both endogenous and exogenous antioxidants, as well as effective antioxidant enzymes [3]. The involvement of HIV or its virulent proteins in the perturbation of cellular antioxidant enzymes activities has not been decisive. However, some reports have shown a decrease in activity of the antioxidants enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione oxidase (GPx) activities have been reported to be lower in HIV-infected individuals [8,9]. Depletion of circulatory endogenous antioxidants, including reduced glutathione (GSH) and vitamins have also been observed in HIV-infected patients, and the lower antioxidant levels correlate with low $CD4^+$ counts [9,10]. A recent study from our research group reported that homozygous deletion of GSTM1 and GSTT1 genes has no effect on total GST activity in the serum of HIV-infected individuals, and the observed enzyme activity was attributted to a compensatory mechanism due to other enzymes that play a role in metabolising xenobiotics [11]. Even though it was expected in the previous study that a double deletion of the GST polymorphic gene will result in a decrease in the total GST enzyme activity, and play a role in disease conditions such as cancers [12,13], the contrary finding suggests that antioxidant enzymes activities in HIV patients may be influenced by other factors such as race, geographical location and trace elements.

A number of antioxidant enzymes are metalloenzymes and depend on trace elements for their activities. Trace elements availability is therefore crucial for effective enzymatic activities. The involvement of trace elements has been shown to be key in the activities of super oxide dismutase (SOD) and demonstrated in the three isoforms of the enzyme: a cytosolic copper/zinc form (CuZnSOD), a manganese form (MnSOD) in the mitochondrion, and an extracellular isoform (EC-SOD) which is found in plasma [14].

This current study is based on the premise that antioxidant enzymes activities and trace element levels in Ghanaian HIV patients may differ from what have been reported. We hypothesize that HIV/AIDS disease progression marked by reduced CD4⁺ count will be associated with significantly reduced antioxidant enzymes activities and trace element levels in Ghanaian patients. The study therefore aimed at determining the effect of ART on antioxidant enzymes activities and trace elements levels in Ghanaian HIV patients to establish their relationship with viral replication and/or CD4⁺ count.

Materials and methods

Study population and sampling site

Study participants were recruited from the Fevers Unit of the Korle Bu Teaching Hospital, a national referral hospital in Accra, Ghana. The study was approved by the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (Study approval number 068/14-5), and all participants signed a consent form after the objectives of the study have been explained to them. A total of 242 participants, comprising of 105 HIV-infected patients on ART, 77 HIV-infected ART-naïve, and 60 HIV seronegative individuals were recruited for the study over a period of six months from September 2015 to February 2016. The sample size calculation was based on a bigger research project which was designed to investigate glutathione S-Transferase (GST) gene polymorphism and antioxidant enzymes activity in HIV/AIDS progression in Ghanaian patients [11], of which this study was part. A prevalence of 30%, a

Margin of Error of 6%, and a Confidence Interval Score of 1.96 based on a Confidence level of 95%. The calculation resulted in a sample size of 224 subjects. The study recruited patients who are HIV positive with or without ART but not taking other medications, multivitamins, or mineral supplements for at least 3 months prior to the collection of blood samples. Patients who have been diagnosed with hepatitis/opportunistic infections, and those who consumed alcohol, smoked, had diabetes or kidney problems, had liver dysfunction, and pregnant women were excluded from the study. Blood samples were collected from each recruited individual under aseptic conditions and were divided into ethylenediaminetetraacetic acid (EDTA) and gel separator tubes. The whole blood in EDTA tubes was used for haematological profiling, and CD4⁺ count and trace elements levels determinations. Blood samples in gel separator tubes were kept on ice and allowed to clot, and sera obtained by centrifugation were kept frozen at -20 °C until ready for analyses. The sera were used for antioxidants estimation, antioxidant enzymes activities, as well as liver function tests. Demographic data of the patients were obtained using standard questionnaire, and patients' treatment regimen was retrieved from medical records after permission has been given by the hospital authority.

Haematological profile and CD4⁺ Count

Full blood cell count was determined with an ABX Pentra 120 DX automated haematology analyser (Horiba ABX, Montpellier, France), and CD4⁺ count was estimated by a BD FACS machine (BD, Brussels, Belgium).

Liver function tests

Serum albumin, total protein, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transferase (GGT) were measured using an automated Biosystems A25 Chemistry Analyser (Madrid, Spain), following the manufacturers' instructions.

Super oxide dismutase (SOD)

Super oxide dismutase (SOD) activity was determined using a commercial kit from Sigma-Aldrich (Missouri, USA) following the manufacturer's protocol. The protocol required that 20 μ L of sample solution is added to each sample and a well which is labelled as blank 2, whilst 20 μ L of double distilled water was added to two wells that are labelled as blanks 1 and 3. A volume of 200 μ L WST Working Solution was added to each well and mixed. Dilution Buffer of 20 μ L was added to blanks 2 and 3, and 20 μ L of Enzyme Working Solution was added to the samples and blank 1. The reaction mixture was mixed thoroughly and incubated at 37°C for 20 minutes. Absorbance was read at 450 nm using a Varioskan Lux microplate reader (Thermo Scientific, Vantaa, Finland), and SOD activity was calculated as described by the manufacturer. The SOD activity assays were done in duplicates.

Glutathione reductase (GR) activity

The sample for the glutathione reductase (GR) activity assay was prepared using the method adopted by Buizza et al. [15]. The serum samples were pre-treated to destroy GSH by adding 5 μ L of 3% H₂O₂ to 100 μ L of the sample and incubated at 25 °C for 5 minutes. A volume of 5 μ L catalase was added to the reaction mixture and incubated under the same conditions of temperature and incubation time as the pre-treatment. The catalase-treated reaction mixture was used to determine the GR activity using a commercial kit (Abcam, Cambridge, UK). Briefly, 20 μ L of the reaction mixture was made up to 50 μ L with assay buffer in a 96-well plate,

and a 50 µL mix reagent (40 µL GR assay buffer, 2 µL DTNB solution (Ellman's reagent), 2 µL NADPH-GNERAT solution and 6 µL GSSG solution) was added to each well to a final volume of 100 µL. Absorbance at 405 nm (A1) and time (T1) were taken immediately after the addition of the mix reagent. The reaction was incubation at 25 °C in the dark for 10 minutes (T2) and the absorbance (A2) was taken again. The change in absorbance (ΔA_{405nm}) was calculated as A2 –A1. A standard curve for TNB was plotted as described by the protocol, and the ΔA_{405nm} value for each sample was extrapolated from the standard curve to obtain equivalent change in TNB (ΔB). The GR activity was calculated as described by the manufacture.

Reduced glutathione (GSH) levels

Total Glutathione (GSH+GSSG) was measured using an assay kit (Sigma-Aldrich, Missouri, USA), following the manufacturer's protocol. Briefly, plasma samples were deproteinized using 5% 5-sulfosalicylic acid, and 10 μ L of each sample was added to 150 μ L working mixture reagent in a 96 well-plate and incubated at room temperature for 5 minutes. A volume of 50 μ L NADPH working solution (0.16 mg/mL) was added to each well and mixed thoroughly, and the plates were read at 405 nm with kinetic read at 1-minute intervals for 5 minutes. A standard curve for GSH was plotted as described by the protocol and Δ A405/min was extrapolated from the curve. The amount of reduced GSH was calculated as described by the manufacturer.

Estimation of trace elements in whole blood

Trace elements were estimated using atomic absorption spectrometry. Briefly, 2 g of blood samples were digested in a mixture of 6 mL of 65% nitric acid and 1 mL of 30% hydrogen peroxide in polytetraflouroethylene (PTFE) Teflon bombs using milestone microwave labstation ETHOS 900, INSTR: MLS-1200 MEGA. Each of the digested samples was made up to 20 mL with double distilled water and assayed for the presence of Iron (Fe), Manganese (Mn), Selenium (Se) and Copper (Cu) using VARIAN AA 240FS-Atomic Absorption Spectrometer in an acetylene-air flame (FLUKA ANALYTICAL, Sigma-Aldrich Chemie GmbH).

Statistical analysis

Parametric data were represented as mean \pm standard deviation, and One-way ANOVA was used to compare the groups. Pearson's correlation coefficient was determined between oxidative stress markers, CD4⁺ count, and trace elements. GraphPad Prism version 6 was used for all analyses, and *p*-values < 0.05 were considered as statistically significant.

Results

Socio-demographics and patients treatment regimen

Females participants were more than males, whilst the control group $(38.7 \pm 1.9 \text{ years})$ and ART-naïve patients $(41.1 \pm 1.4 \text{ years})$ were younger than the patients on ART $(45.6 \pm 0.9 \text{ years})$ (p ≤ 0.01). There was however no statistically significant difference between the ages of the control group and the ART-naïve patients (p > 0.05). The demographics of the patients with respect to sex and age have been reported in a previous paper [11]. The average amount of time the subjects have been on ART is approximately 3.7 years.

The antiretroviral drugs that were used by the patients on ART in various combinations included Lamivudine (3TC), Zidovudine (AZT), Efavirenz (EFV), Nevirapine (NVP), Atazanavir (ATV), and Lopinavir (LPV). The combinations were 3TC-EFV, 3TC-NVP, 3TC-AZT-EFV, 3TC-AZT-NVP, 3TC-AZT-ATV, 3TC-AZT-LPV, 3TC-TDF-EFV, 3TC-TDF-EFV, 3TC-TDF-NVP, 3TC-TDF-NVP-ATZ, 3TC-TDF-LPV and 3TC-LPV.

Haematological profile and trace elements

Individuals recruited for the study comprised of HIV seropositive patients on ART and ARTnaïve, and HIV seronegative controls. ART-naïve patients showed altered haematological indices when compared with patients on ART and the seronegative controls. Low levels of haemoglobin (HB), hematocrit, mean cell volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were observed in the ART-naïve patients compared to the ART and seronegative groups (p < 0.001). Conversely, white blood cells (WBC), red blood cells (RBC), neutrophils and lymphocytes levels were significantly elevated in the ART-naïve patients compared to the patients on ART (p < 0.001) (Table 1). The ART-naïve patients also showed low levels of trace elements (iron, manganese, copper and selenium) than the patients on ART and the seronegative group (p < 0.001) (Table 1). There were increasing levels of the haematological indices and trace elements with increasing CD4⁺ count in HIV seropositive patients (Table 2).

Liver function tests

The enzymatic activities of ALP, AST and GGT, and the levels of albumin and total protein were significantly elevated in the HIV-positive individuals compared to seronegative controls [*p*-value < 0.001] (Table 3). ALP activity and total protein levels in ART-naïve patients were significantly higher, whereas albumin level was significantly lower in the ART-naïve group compared to those on ART (*p*-value < 0.05). Activities of ALP, AST and GGT in the HIV-positive individuals were decreased with increasing CD4⁺ count, whereas albumin level significantly increased with increasing CD4⁺ count (Table 4).

Glutathione reductase (GR) activity

ART-naïve patients showed significantly lower GR activity compared to patients on ART and seronegative controls (p-value < 0.001) (Fig 1A), and the activity increased with increasing

Parameter	Pa	Patients		ANOVA <i>p</i> -value	
	ART (N = 105)	ART-naïve (N = 77)	(N = 60)		
HB (g/dL)	$12.70 \pm 0.15^{\phi}$	$10.79 \pm 0.25^{*}$	12.80 ± 0.23	< 0.001	
RBC (10 ¹² /L)	$3.84 \pm 0.05^{\phi_{*}}$	$4.05 \pm 0.09^{*}$	4.51 ± 0.08	< 0.001	
WBC (10 ⁹ /L)	$4.82 \pm 0.13^{\phi}$	$5.59 \pm 0.40^{*}$	3.05 ± 0.49	< 0.001	
Hematocrit (%)	$37.98 \pm 0.48^{\phi_*}$	$32.70 \pm 0.70^{*}$	42.22 ± 0.66	< 0.001	
MCV (f)	$100.00 \pm 1.21^{\phi*}$	$81.15 \pm 0.96^{*}$	94.00 ± 1.13	< 0.001	
MCH (pg)	$33.36 \pm 0.39^{\phi*}$	26.17 ± 0.35*	28.46 ± 0.38	< 0.001	
Neutrophils (10 ⁹ /L)	$1.25 \pm 0.08^{\phi*}$	$1.37 \pm 0.14^{*}$	0.83 ± 0.06	< 0.001	
Lymphocytes (10 ⁹ /L)	$3.31 \pm 0.11^{\phi_*}$	$3.44 \pm 0.19^{*}$	1.28 ± 0.08	< 0.001	
Iron (µg/dL)	$51.12 \pm 17.46^{\phi_*}$	31.05 ± 15.71*	80.57 ± 17.81	< 0.001	
Manganese (µg/dL)	$63.23 \pm 10.84^{\phi*}$	25.98 ±11.02*	76.21 ± 10.45	< 0.001	
Copper (µg/dL)	$35.16 \pm 14.28^{\phi_*}$	23.01 ±14.97*	42.84 ± 13.54	< 0.001	
Selenium (µg/dL)	$4.32 \pm 1.05^{\phi_*}$	$2.88 \pm 0.72^{*}$	6.20 ± 1.37	< 0.001	

Table 1. Haematological and trace elements profiles of HIV patients' groups and the seronegative individuals.

p-values were obtained from One-way ANOVA by comparing the HIV patient groups with the seronegative controls. Hb: hemoglobin, RBC: red blood cells, WBC: white blood cells, MCV: Mean Cell Volume, MCH: Mean Corpuscular Hemoglobin. Bonferroni multiple comparison was used to compare the three groups. *mean value compared with seronegative group.

 $^{\phi}$ mean value compared between ART and ART-naïve groups. Parameters were presented as mean \pm SD.

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Parameter		ANOVA		
	< 200 (n = 37)	200-350 (n = 31)	>350 (n = 114)	<i>p</i> -value
HB (g/dL)	$10.36 \pm 0.46^{\phi}$ *	11.96 ± 0.36	12.38 ± 1.54	< 0.001
RBC (10 ¹² /L)	$3.86 \pm 0.14^{\phi}$ *	3.98 ± 0.13	3.94 ± 0.06	< 0.001
WBC (10 ⁹ /L)	$4.60 \pm 0.27^{\phi}$ *	5.28 ± 0.90	5.28 ± 0.14	< 0.001
Hematocrit (%)	$31.51 \pm 1.30^{\phi}$ *	$35.96 \pm 0.99^*$	37.10 ± 0.45	< 0.001
MCV (fL)	$82.24 \pm 1.37^{\phi}$ *	$90.50 \pm 2.20^{*}$	95.62 ± 1.39	< 0.001
MCH (pg)	$26.64 \pm 0.50^{\phi}$ *	$29.07 \pm 0.93^*$	31.86 ± 0.46	< 0.001
Neutrophils (10 ⁹ /L)	$1.22 \pm 0.15^{\phi}$ *	1.36 ± 0.26	1.31 ± 0.09	< 0.001
Lymphocytes (10 ⁹ /L)	$2.71 \pm 0.22^{\phi*}$	$3.00 \pm 0.36^{*}$	3.65 ± 0.11	< 0.001
Iron (μg/dL)	29.56 ± 20.13*	38.63 ± 18.30*	53.12 ± 20.74	< 0.001
Manganese (µg/dL)	26.39 ±14.82 ^{\$\$}	56.19 ± 15.40*	64.01 ±14.23	< 0.001
Copper (µg/dL)	$21.24 \pm 14.63^{*}$	27.95 ± 16.38*	39.07 ± 13.46	< 0.001
Selenium (µg/dL)	$2.19 \pm 1.17^{*}$	3.02 ± 1.83*	4.67 ± 1.81	< 0.001

Table 2. Haematological and trace elements profiles of the HIV patients stratified by CD4⁺ counts.

p-values were obtained from One-way ANOVA by comparing the CD4⁺ count stratification. Hb: hemoglobin, RBC: red blood cells, WBC: white blood cells, MCV: Mean Cell Volume, MCH: Mean Cell Hemoglobin. Bonferroni multiple comparison was used to compare the three groups.

*mean value was significantly altered as compared with the group showing $CD4^+$ count >350, p < 0.001.

⁶mean value was significantly altered compared with the group showing CD4⁺ count from 200–350. Parameters were presented as mean ± SD. n is the number for the subgroups.

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CD4⁺ count (*p*-value < 0.001) (Fig 1B, Table 5). The trace elements had a weak correlation with GR activity (Table 5).

Reduced glutathione (GSH) level

Reduced glutathione (GSH) level of ART-naive patients was significantly lower than patients on ART and the seronegative controls (p<0.0001) (Fig 1C), and the level increased with increasing CD4⁺ count (p<0.001) (Fig 1D, Table 5). As with the GR activity, the trace elements also had a weak correlation with GSH level (Table 5).

Table 3. Liver function test results of the of HIV patients' groups and the seronegative individuals.

Parameter	HIV-infected participants		Control (N = 60)	ANOVA
	ART (N = 105)	ART naïve (N = 77)		<i>p</i> -value
ALP (U/L)	216.32 ±127.90 [¢] *	271.11 ± 155.5*	149.01± 67.30	< 0.001
ALT (U/L)	19.93 ±10.01	21.93 ± 11.83	20.82 ± 11.50	0.480
AST (U/L)	39.51 ± 16.93*	$42.87 \pm 23.15^*$	27.00 ± 11.20	< 0.001
GGT (U/L)	68.58 ± 33.12*	$60.68 \pm 31.83^*$	38.30 ± 14.17	< 0.001
Total protein (g/L)	$97.46 \pm 14.57^{\phi*}$	104.58 ± 13.55*	72.93 ± 16.31	< 0.001
Albumin (g/L)	$45.54 \pm 8.96^{\phi*}$	38.89 ± 6.88	37.91 ± 5.22	< 0.001

p-values were obtained from One-way ANOVA by comparing the HIV patient groups with the seronegative controls. ALP = Alkaline phosphatase; ALT = Alanine transaminase; AST = Aspartate transaminase; GGT = Gamma glutamyl transferase. Bonferroni multiple comparison was used to compare the three groups. *mean value compared with seronegative group.

 $^{\phi}$ mean value compared between ART and ART-naïve groups. Parameters were presented as mean \pm SD.

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Table 4. Liver function tests in HI	V patients stratified by CD4+ counts.
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Parameter		ANOVA			
	<200 (n = 37)	200-350 (n = 31)	>350 (n = 114)	<i>p</i> -value	
ALP (U/L)	$333.21 \pm 157.51^{\phi_{*}}$	257.60 ± 143.91*	204.14 ±116.93	< 0.001	
ALT (U/L)	$29.32 \pm 13.22^{\phi*}$	17.90 ± 9.92	18.80 ± 8.12	< 0.001	
AST (U/L)	53.51 ± 36.80*	40.30 ± 27.20	37.01 ± 17.14	0.002	
GGT (U/L)	$84.40 \pm 14.12^{\phi_*}$	72.50 ± 24.13*	57.13 ± 5.52	< 0.001	
Total protein (g/L)	101.61 ± 19.82	101.50 ± 11.53	99.83 ± 14.64	0.764	
Albumin (g/L)	36.73 ± 8.54*	$40.94 \pm 7.41^*$	45.24 ± 8.21	< 0.001	

p-values were obtained from One-way ANOVA by comparing the CD4⁺ count stratification. ALP = Alkaline phosphatase; ALT = Alanine transaminase;

AST = Aspartate transaminase; GGT = Gamma glutamyl transferase. Bonferroni multiple comparison was used to compare the three groups.

*mean value of the group was significantly altered as compared with other groups, p < 0.001.

 $^{\phi}$ mean value was significantly altered compared with the group showing CD4⁺ count from 200–350. Parameters were presented as mean ± SD. n is the number for the subgroups.

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Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was significantly reduced in ART-naïve patients compared to those on ART and the control group (*p*-value <0.0001), but there was no significant difference between the SOD levels for the patients on ART and the seronegative control group (*p*-value > 0.05) (Fig 1E). SOD activity in patients with CD4⁺ count <200 cells/mm³ was significantly decreased when compared with patients with CD4⁺ count >350 cells/mm³, (*p*value < 0.02) (Fig 1F). There was also a weak correlation between the trace elements and SOD, except for manganese which showed a strong negative correlation with SOD activity (*p*value < 0.01) (Table 5).

Discussion

Human immunodeficiency virus (HIV) infection has been reported to induce high levels of reactive oxygen species (ROS) in seropositive individuals. The observed disease complications in patients have been attributed to the effects of ROS on tissues [3]. The current study investigated the effect of antiretroviral therapy (ART) on antioxidant enzyme activities and trace elements levels in Ghanaian HIV patients.

Antioxidant enzymes activities are low in ART-naïve HIV-infected patients and high in those on ART. This observation comes from a significant decreased in SOD and GR activities, and GSH levels in the ART-naïve patients compared to patients on ART and seronegative controls. The finding from this study agrees with other studies that have reported reduced antioxidant enzymes activities in HIV-infected patients; ART-naïve individuals showed reduced blood SOD, glutathione oxidase (GPx), catalase (Cat) activities, and elevated malondialdehyde levels compared to healthy controls [4,16]. Increase in antioxidant enzymes activities has been shown to improve the health conditions of HIV positive patients, and antioxidant supplementation was found to decrease lipid peroxidation and improve CD4⁺ counts in ART-naïve HIV-infected patients [17]. Glutathione reductase (GR) is responsible for converting oxidized glutathione (GSSG) to the reduced form (GSH), and therefore low GR activity will lead to elevated GSSG levels, which will result in increased oxidative stress and induce viral replication [5,18]. Administration of ART in HIV management has been shown to induce oxidative stress in patients, however, ART induced oxidative stress is minimal, compared to the level of ROS generated by the viral infection in patients [19–21]. The mechanistic role of oxidative stress in



Fig 1. A-F. Effect of CD4⁺ count and treatment options on the levels of reduced GSH and antioxidant enzymes activity in HIV-positive patients compared with seronegative group. Super oxide dismutase (SOD) activity and total glutathione (GSH+GSSG) were determined using commercial kits, and glutathione reductase (GR) activity assay was performed using the method adopted by Buizza et al.

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viral replication is through the induction of host transcriptional factor Hypoxia-Inducible Factor-1 (HIF-1) by the HIV protein Vpr to increase mitochondrial activity, resulting in ROS production which activates HIV-1 LTR [22,23].

Manganese seems an important trace element for HIV disease management. The conclusion stems from the fact that the level of manganese was strongly and positively associated with CD4⁺ count. However, the other trace elements, Fe, Se, and Cu, showed a weak correlation with CD4⁺ count, GSH levels, and GR and SOD activities. Almost two decades ago, it was shown that elevated concentrations of manganese inhibited reverse transcription *in vivo* through occupancy of the B site of reverse transcriptase [24]. However, a search through literature did not reveal manganese as a constituent of antiretroviral drugs. Meanwhile the trace element has also been implicated to increase mutation rate in HIV-1 [25]. More work is therefore

Parameters	CD4 ⁺ count	SOD	GR	GSH	Se	Cu	Fe	Mn
CD4 ⁺ count	1	0.073	0.187*	0.176*	0.110	0.098	0.045	0.203**
SOD	0.073	1	0.181**	0.308**	-0.013	-0.100	-0.048	-0.241**
GR	0.187*	0.181**	1	0.247**	-0.013	0.030	-0.019	0.009
GSH	0.176*	0.308**	0.247**	1	0.077	-0.003	0.076	-0.039
Se	0.110	-0.013	-0.013	0.077	1	0.242**	0.677**	0.236**
Cu	0.098	-0.100	0.030	-0.003	0.242**	1	0.026	0.492**
Fe	0.045	-0.048	-0.019	0.076	0.677**	0.026	1	-0.012
Mn	0.203**	-0.241**	0.009	-0.039	0.236**	0.492**	-0.012	1

Table 5. Pearson's correlation coefficients of CD4⁺ count, antioxidant enzymes activities and trace elements in HIV patients.

SOD; superoxide dismutase, GR; glutathione reductase, GSH; reduced glutathione, Se: Selenium, Cu; Copper, Fe; Iron, Mn; Manganese. P values *p < 0.05

***p*< 0.01

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needed to understand the role of manganese in the improvement of CD4⁺ count in HIV patients.

Stratification analysis revealed higher levels of trace elements in patients with CD4⁺ counts greater than 350 cell/mm³, and suggests that the elements are important in improving the health conditions of the patients. In keeping with the results from this study, earlier studies have shown significant reduction in trace elements levels in HIV-infected patients with high viral loads [26–28]. The SOD activity measured in this study included the activities of CuZn-SOD and MnSOD, and interestingly a negative correlation between manganese level and total SOD activity was observed. It has been established that HIV Tat protein down regulates the synthesis and induction of MnSOD [29–31]. The decreased SOD activity observed in the HIV patients may be attributed to the inhibition of MnSOD by HIV Tat protein and resulting in the negative correlation between Mn level and SOD activity.

ART-naïve HIV-infected patients were anaemic. This study showed a decreased level of haemoglobin (HB) in ART-naïve patients compared to patients on ART and the seronegative controls. The levels of HB decreased with low CD4+ counts in the HIV-infected individuals and supports previous reports which suggest that anaemia in HIV-infected patients is associated with poor disease outcomes [32–34]. The normal haematological indices observed in patients on ART as compared to the seronegative controls shows a response to treatment and a reduction in oxidative stress-induced red blood cells damage [35,36].

The study showed decreased levels of hemoglobin (HB), hematocrit, mean cell volume (MCV) and mean cell hemoglobin (MCH), with white blood cells (WBC), neutrophils and lymphocytes significantly elevated in the ART-naïve patients than the patients on ART and the control group. The low hematological indices together with the observed low Fe²⁺ levels suggest a microcytic anaemia in the ART-naïve HIV-infected patients and support previous studies that have reported low HB, MCV and MCH in HIV treatment-naïve patients compared to patients on ART [32, 33]. The increased levels of WBC, neutrophils and lymphocytes have been implicated in infection-associated complications [34], and consistent with elevated immune response due to high viral load in the ART-naïve patients. The levels of HB, MCV and MCH decreased with low CD4⁺ counts in the HIV-infected individuals and supports previous reports which suggest that anaemia in HIV-infected patients is associated with poor disease outcomes [35,36]. The low lymphocyte count in patients with low CD4⁺ count confirms that the HIV infection targets T-cells. The normal haematological indices observed in patients and a

reduction in red blood cells damage. These observations are in agreement with previous reports [37,38].

The liver plays an important role in detoxification or clearance of ROS through metabolism of compounds which potentially generate ROS and the synthesis of antioxidant enzymes [39]. Liver toxicity was observed to be higher in the ART-naïve HIV-infected patients than those on ART and seronegative controls. The levels of alkaline phosphatase (ALP), aspartate transferase (ASP) and gamma glutamyl transferase (GGT) were significantly elevated in the ART-naïve patients. Even though ART has been implicated in liver damage [40], the increased levels of markers of hepatotoxicity in ART-naïve patients as compared to those on ART could be attributed to the HIV infection exclusively. Advanced stages of HIV/AIDS indicated by reduced levels of CD4⁺ count in patients reported in this study, showed a corresponding elevated markers of hepatotoxicity. HIV-infected patients without any risk factors of liver damage have been shown to have elevated alanine aminotransferase (ALT) level, and this suggests that the infection is the underlying cause of the increased activities of liver enzymes [41,42].

Conclusion

We observed that antioxidant enzymes activities and trace elements levels were decreased in ART-naïve HIV-infected patients compared to those on ART and increase in manganese level correlates positively with CD4⁺ counts, suggesting that the trace element is important for better disease outcome in HIV patients. The study suggests that ARTs are helpful in the increase of antioxidant enzymes activities in HIV patients. More studies should also be done on manganese to ascertain how the trace element improves CD4⁺ count in HIV patients. Routine monitoring of antioxidant levels or enzymes activities of patients infected with HIV should be done as part of HIV management.

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