Inflammatory, Oxidative Stress Status and Antioxidant Homeostasis in HIV-Infection and Ageing are Tightly Logistic in Humans

ABSTRACT

Aims: Inflammatory, immunological, oxidative stress status and antioxidant homeostasis in HIV-Infection and ageing were studied to determine if their relationships were logistic in humans.

Study design: The effect of HIV disease/antiretroviral therapy on Inflammatory, immunological, oxidative stress status and antioxidant homeostasis was assessed by juxtaposition with similar indices in HIV seronegative apparently healthy young (20-35years) adults (CTRL) and HIV seronegative elderly (65-86 years) subjects (ELD65+).

Place and Duration of Study: Subjects include One hundred (100) HIV seropositive individuals, 50 on ART and 50 ART-Naive and One hundred (100) seronegative individuals comprising fifty (50) healthy younger adults and Fifty (50) elderly (≥65yrs) individuals attending the Federal Medical Centre Owerri between August and December 2020.

Methodology: Venous blood was collected into an EDTA vacutainer and plain vacutainer plain from each participant for inflammation indices (ESR, CRP) using Westergren and Finecare CRP rapid quantitative test kit, Oxidative stress index (erythrocyte lipid peroxidation as thiobarbituric acid reactive substances (TBARS), antioxidant index (glutathione(reduced) using spectrometric method were determined on subjects. Enzyme-.linked immunosorbent assay (ELISA) was used to determine interleukin-6 (IL-6). The data generated was analysed by a one way analysis of variances (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21; Relationship existing between parameters were analysed using table curve 2D (Systat USA).

Results: Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) Interleukin-6 (IL-6) and thiobarbituric acid reactive substances (TBARS) were all significantly higher in seropositive young adult subjects ($P<0.05$) than the seronegative young adult control subjects. Glutathione (GSH) was significantly reduced in the seropositive young adult subjects ($P<0.05$) than the seronegative young adult control subjects. In all the parameters measured, Naïve and ART subjects were similar in trend to ELD65+ subjects suggesting immune ageing. The antioxidant parameter GSH had an inverse relationship with the inflammatory (ESR, CRP and IL-6) and oxidative stress (TBARS) parameters. This relationship was logistic and followed logistic dose response relationship and a sigmoidal association.

Conclusion: We conclude that Erythrocyte sedimentation rate, C-reactive protein, Interleukin-6 (IL-6), Glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) are useful parameters to assess immune ageing, and are related logistically in humans

Keywords: HIV, immune ageing, C-reactive protein, interleukin-6, erythrocyte sedimentation rate, glutathione, logistic dose response.

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) is a lentivirus of the retrovirus family. They are RNA containing viruses that replicate with the help of the reverse transcriptase (RT) or RNA dependent DNA polymerase (Vasudevan *et al.,* 2016). HIV infection is associated with a chronic inflammatory state arising from multiple factors, including innate immune recognition of HIV, increased microbial translocation, and release of endogenous ligands from damaged cells (such as CD4 T cells). In many respects, this increased pro-inflammatory environment bears a resemblance to that associated with ageing in the absence of HIV infection (Zapata and Shaw, 2014). These conditions in HIV-infections distort the antioxidant systems and resulting to inflammation giving rise to increased damage to cell and peroxidation. There is age-related shrink in immune functions, referred to as immunosenescence, which is partially responsible for the increased prevalence and severity of infectious diseases.

A great number of reactions scheduled in cells are coupled with transfer of redox equivalents. So, maintenance of a particular redox state in cytoplasm is an important condition for the normal life of the cell. Both redox activity of glutathione (GSH) with its resistance to auto-oxidation and high concentration and its ability to maintain its reduced state make it the most important intracellular redox buffer. Age-associated inflammation, or "inflammaging," is a major risk factor for both morbidity and mortality in older adults (Montecino-Rodriguez *et al.,* 2013). Chronic inflammation not only impacts the functioning of the immune system, but also contributes to an increased prevalence of many diseases in the general. C-reactive protein (CRP) could help to restore homeostasis and reduce microbial growth independently of antibodies during trauma, stress, or infection (Chen *et al.,* 2018). Its concentration is now used as a marker of inflammation. Erythrocyte sedimentation rate is also used as a non specific but an adjunct marker of inflammation. Glutathione (reduced) is the single most important parameter to assess antioxidant homeostasis, while thiobarbituric acid reactive substances (TBARS) which is employed to assess the concentration of malondialdehyde (a product of lipid peroxidation) captures extent of oxidative damage to macromolecules including lipids of the cell membranes.

Alisi et al., 2011 showed that the relationship that exists between antioxidants, oxidative damage to lipids (peroxidation) and the delicate balance between the activities and the intracellular concentrations of antioxidants in rats were tightly logistic. We wish to show that response of the antioxidant glutathione which is the most important intracellular redox buffer to oxidative damage in HIV and ageing is logistic. This tight logistic association is here assessed in inflammation, peroxidation and antioxidant homeostasis in HIV-infection/ Antiretroviral therapy, Ageing and Health. It is our aim to assess if disequilibrium in Inflammatory, Oxidative Stress Status and Antioxidant Homeostasis in HIV-Infection and Ageing is tightly logistic in humans.

2. MATERIAL AND METHODS

2.1 Study Area

This study was carried out at Federal medical Centre Owerri in Imo State, Nigeria. Imo state is one of the 36 states of Nigeria and is located in the South Eastern Zone between latitude 4o45oN and 7o15'N, longitude 6o50o'E and 7o25'E. This hospital is one of the tertiary referral centres which provide adequate medical care to HIV-infected individuals in Nigeria through the heart-to-heart Clinic and sick individuals at large.

2.2 Study Population and Sample Size

It was a cross sectional study and was conducted prospectively among patients attending the heart-to-heart and while the other participants were drawn from the General out-patient department (GOPD) of the Federal Medical Centre Owerri and the populace. The minimum

sample size was obtained using the formula by Naing et al., 2006. Prevalence rate of seropositive in the South Eastern Nigeria is 1.9% (NACA 2019)

2.3 Study Design

The study was carried out on four groups.
Group1 = 50 HIV seropositive subjects (age 20 to 35) ART-naive (NAIVE)
Group 2 = 50 HIV seropositive subjects (age 20 to 35) on ART (ART)
Group 3 = 50 HIV seronegative control subjects (age 20 to 35) (CTRL)
Group 4 = 50 seronegative Elderly (>65yrs) control subjects (ELD65+) served as CTRL 2
An informed consent was extracted from the subjects. There was an absolute assurance of confidentiality of the patient.

2.3.1 Selection criteria

The subjects were selected under defined criteria.
HIV seropositives subject included in this study are generally 20-35years old. Those on Anti-retroviral therapy (ART) would have spent at least 3 months on therapy. Pregnant or planning to be pregnant in the next 4 months are excluded. People on traditional, herbal or complementary medicines, people on mind altering medications, and subjects positive for HbsAg and HCV are excluded from the study.
HIV seronegatives: Subjects (CTRL and ELD65+) are generally negative to HIV, HBV and HCV. They are age 20-35years and >65years old respectively. Pregnant or planning to be pregnant in the next 4 months are excluded. People on traditional, herbal or complementary medicines or people on mind altering medications, and people positive for HbsAg and HCV are excluded from the study.

2.5 Blood Sample Collection

About 6mls of venous blood was drawn from the ante cubital vein for haematological and biochemical analysis. About 3mls was put into an EDTA vacutainer for determinations that require whole blood (HIV, HBsAg, HCV, ESR and erythrocyte Glutathione). 3mls was also put into an EDTA vacutainer centrifuged for 5minutes at 3000rpm to separate the plasma. The separated plasma is stored in refrigerator for estimation of thiobarbituric acid reactive substances (TBARS).

2.6 Determination of Erythrocyte Sedimentation Rate (ESR)

The measurement of erythrocyte sedimentation rate was carried out by Modified Westergren Method (National Committee for Clinical Standards, 1993) ESR was set up within six hours after collection of blood using Modified Westergren Method as described by the National Committee for Clinical Standards (1993). Briefly, the pipette was used to add 0.5 ml of 0.85% saline in a labelled 13 x 100 mm test tube. The venous blood specimen was gently mixed with the anticoagulant, 5 to 10 times to allow a complete mix of blood and anticoagulant. A pipetting apparatus was used to fill the Westergren pipette to the "0" mark (± 1 mm) with the diluted blood sample and placed in a perpendicular position in the pipette rack for an hour, exactly when the distance (mm) between the meniscus of the plasma and the top of the erythrocytes was read as the ESR.

2.7 Determination of C - reactive protein (CRP) concentration

The Finecare® CRP rapid quantitative test is a fluorescence immunoassay used along with Finecare FIA system for quantitative determination of CRP in human whole blood, serum or plasma. The Finecare® CRP rapid quantitative test is based on fluorescence immunoassay technology. The Finecare CRP rapid quantitative test uses a Sandwich immunodetection method. When the sample is added into the sample well of the test catridge, the fluorescence-labelled detector CRP antibodies on the sample pad bind to CRP antigens in blood specimen and the form immune complexes. As the complexes migrate on the

nitrocellulose matrix of test strip by capillary action, the complexes of detector antibodies and CRP are captured to CRP antibodies that have been immobilized on test strip. Thus the more CRP antigens in blood specimen, the complexes accumulated on test strip. Signal intensity of fluorescence of detector of antibodies reflect the amount of captured CRP.

2.8 Estimation of Lipid Peroxidation

Lipid peroxidation in the supernatant fractions was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al. (1979) as described by Liu et al. (1990). The results were expressed in malondialdehyde (MDA) formed relative to an extinction coefficient of 1.56 x 10^6 mol/cm.

2.9 Determination of Glutathione Concentration

Reduced glutathione (GSH) was estimated by its reaction with dithio-bis-2-nitrobenzoic acid (DTNB) that gives a yellow coloured complex with absorption maximum at 412 nm (Raja et al., 2007).

2.10 Interleukin-6 (IL-6) Assay

The commercial Human interleukin 6 (IL-6) ELISA kit of Melsin Medical Co., Limited was used. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay IL-6 in Human serum, blood plasma, urine, and other biological fluids. This was carried out according to the manufacturers' prescriptions. Briefly, standard, test sample and HRP-labeled IL-6 antibodies were added to microtitre wells which are Pre-coated with IL-6 antibody. After incubation and washing to remove the uncombined enzyme, Chromogen Solution A and B was added. The colour of the liquid changed into blue. At the effect of acid, the colour finally becomes yellow. The colour change was measured spectrophotometrically at a wavelength of 450nm. The concentration of IL-6 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.11 Statistical Analysis

Data obtained from the study were analyzed by the use of one-way analysis of variance (anova), all results were given as mean ± sd and values for $p = 0.05$ were considered statistically significant. Relationship between parameters was studied using table 2d curve 5.0 systat usa.

3. RESULTS

3.1 Demographic characteristics of our studied population by State, Sex, and Age, in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV- Seronegative Elderly (>65) Control subjects.

The study population characteristics are as shown (Table 1). Subjects (n=200) drawn from Nigerian (a sub-Saharan Africa population) and comes from different states of the Country including Abia(22), Adamawa(4), Akwa Ibom(2), Anambra(20), Bayelsa(3), Edo(9), Enugu(6), Imo(99), Kaduna(6) and River State(29). Study participant included a total number of 200 subjects and belonging to four groups of fifty subjects each: HIV-Seropositive ART-naïve individuals (NAÏVE); HIV-Seropositive individual on Anti Retroviral Therapy (ART); HIV-Seronegative control subjects (CTRL); and HIV- Seronegative Elderly (≥65) Control subjects (ELD65+). The subjects by Gender included 119(59.5%) males and 81(40.5%) females. The mean ages by group are Naïve (29.52 ± 3.01yrs), ART (30.78 ± 4.63yrs), seronegative control (CTRL) (26.30 ± 3.17yrs) and seronegative elderly controls (ELD65+) (71.37 ± 6.48yrs).

Table 1: Demographic characteristics of studied population

Distribution by State	(n)	PERCENT (%)	NAÏVE	ART	CTRL	ELD65+
ABIA	22	11	6	8	8	0
ADAMAWA	4	2	2	2	0	0
AKWA IBOM	2	1	1	0	1	0
ANAMBRA	20	10	4	3	5	8
BAYELSA	3	1.5	0	3	0	0
EDO	9	4.5	3	3	1	2
ENUGU	6	3	0	2	0	4
IMO	99	49.5	24	18	27	30
KADUNA	6	3	3	2	1	0
RIVERS	29	14.5	7	9	7	6
TOTAL	200	100	50	50	50	50
GENDER						
Male	119	59.5	20(40)	43(86)	21(42)	35(70)
Female	81	40.5	30(60)	7(14)	29(58)	15(30)
AGE(yrs)						
Mean ± SD			29.52 ±3.01	30.78 ±4.63	26.30 ±3.17	71.37 ±6.48
Median			30	33	26.0	69.5
Mode			30	35	27	65
Min-Max			22-35	20-35	20-35	65-86

3.2 Erythrocyte Sedimentation rate (ESR) in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV-Seropositive Elderly (>65) Control subjects

The result of ESR estimation (table 2) in HIV seropositive and seronegative individuals showed that ESR was significantly ($P<0.05$) elevated in HIV seropositive subjects (NAÏVE and ART)(49.16 ± 5.49mm/hr and 24.12 ±2.88mm/hr) as well in the ELD65+(24.28 ±1.77mm/hr)when compared to CTRL (7.66 ± 0.61mm/hr). ART treatment resulted in a significant ($P<0.05$) decrease in ESR among seropositives such that ESR in ART did not significantly ($P>0.05$) vary from those obtained for ELD65+ subjects.

Table 2: Inflammation status in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV- Seronegative Elderly (65+) Control subjects.

INFLAMMATION STATUS	NAÏVE	ART	CTRL	ELD65+
ESR (mm/hr) ± SEM	49.15[a] ±5.49	24.12[b] ±2.88	7.66[c] ±0.61	24.28[b] ±1.77
Median values	34.0	15.5	7.0	20.0
(Min-Max)	(12-150)	(3-95)	(1-20)	(9-63)
(%) within normal range (0-10mm/hr)	0%	22%	76%	2%
(%) outside normal range (>10mm/hr)	100%	88%	24%	98%
(%) outside ranges (>20mm/hr)	86%	42%	2%	56%

CRP (mg/L) ± SEM	41.81[a] ±3.91	17.22[b] ±1.62	5.49[c] ±0.50	20.78[b] ±1.34
Median values	33.6	14.5	4.4	18.0
(Min-Max)	(12.3-115.6)	(2-56.3)	(1.5-17.8)	(8.8-48.0)
(%) within normal range (0-10mm/hr)	0%	26%	88%	2%
(%) outside normal range (>10mm/hr)	100%	74%	12%	98%
(%) outside ranges (>20mm/hr)	82%	26%	0%	46%

176 *Results are presented as Percentages(%); Mean±SD; Mean±SEM and Ranges*

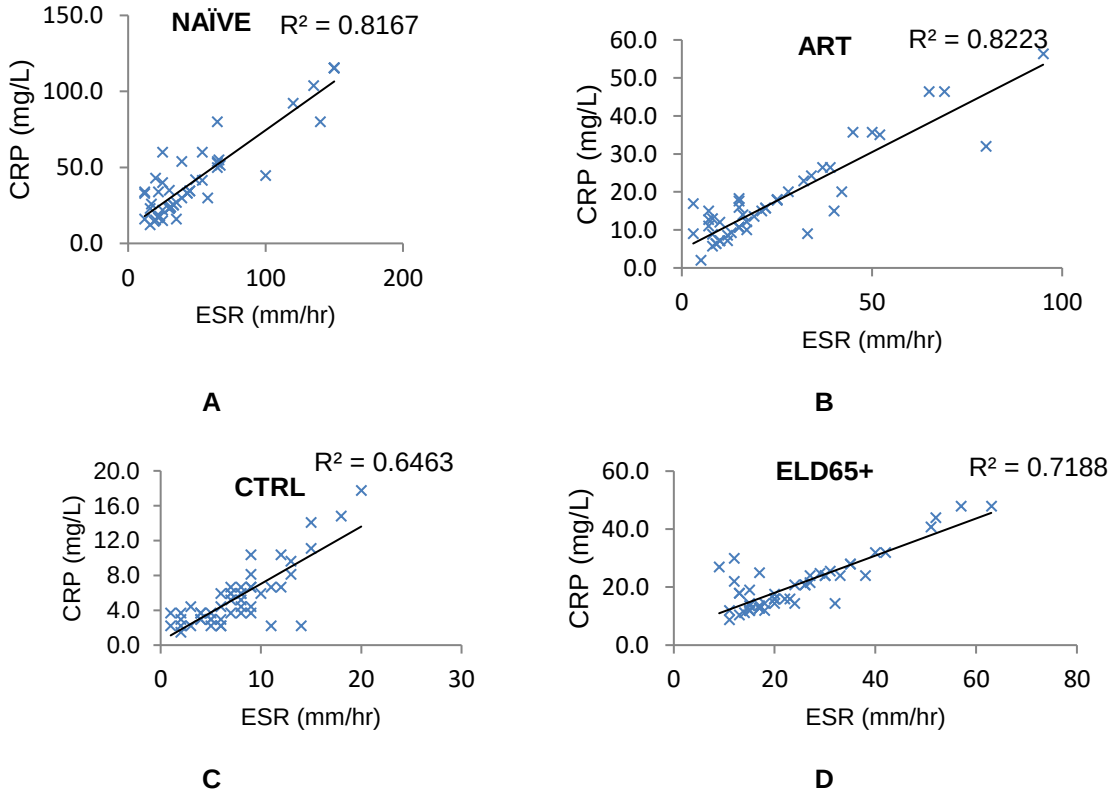

Figure 1. Correlation of Erythrocyte Sedimentation rate with C-reactive Protein in (A) Seropositive ART-naïve individuals (NAÏVE), (B) Seropositive individual on ART (ART), (C) Seronegative control subjects (CTRL) and (D) Seropositive Elderly (>65) Control subjects (ELD65+).

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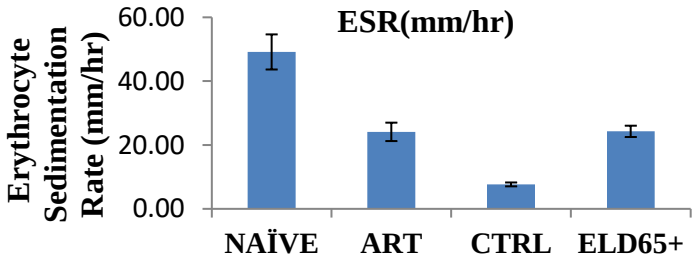

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Figure 1b: Erythrocyte Sedimentation rate (ESR) in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV-Seropositive Elderly (>65) Control subjects.

3.3 C-reactive Protein concentration (mg/L) in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV-Seronegative Elderly (>65) Control subjects

Result showed the CRP estimation (table 2) in CTRL to be 5.5± 3.5mg/l; ELD65+ was 20.8 ± 9.5mg/l; NAIVE was 41.8 ± 27.6mg/l; while the ART was 17.2 ± 11.5mg/l Result showed that CRP concentration was significantly (P<0.05) increased in NAÏVE (41.8 ± 27.6) when compared to CTRL (5.5± 3.5),the ART (17.2 ± 11.5) and ELD65+ (20.8 ± 9.5) subjects. The treatment with ART resulted in a significant (P<0.05) decrease in CRP among seropositives such that CRP in ART did not significantly (P>0.05) vary from those of the ELD65+ subjects.

3.4 Interleukin-6(IL-6) in HIV-Seropositive naïve, Seropositive on ART, Seronegative control and Seronegative Elderly Individuals

The result of serum cytokine, Interleukin-6 (IL-6) concentration (Figure 2) in HIV seropositive and seronegative individuals showed that IL-6 concentration was significantly (P<0.05) elevated in NAÏVE and ART as well in the ELD65+ compared to CTRL. However, ART treatment resulted in a significant (P<0.05) decrease in IL-6 concentration among this seropositive subject, thus IL-6 Concentration in ART treated subjects were similar to those obtained for the elderly subjects. In the groups NAIVE, ART, CTRL and ELD +65, IL-6 concentration was 9.91 ± 3.83, 5.62 ± 1.60, 2.69 ± 0.48 and 5.63 ± 1.76 pg/ml respectively.

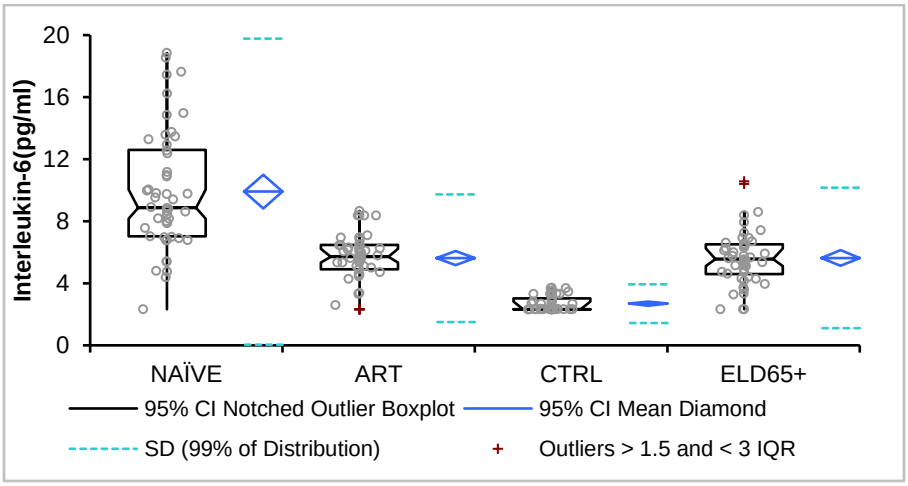

Figure 2: Interleukin-6 Concentrations in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV-Seropositive Elderly (>65) Control subjects.

3.5 Glutathione (GSH) level in HIV-Seropositive naïve, Seropositive on ART, Seronegative control and Seronegative Elderly Individuals

Result of serum GSH concentration (figure 3) showed that GSH in serpositives and the sernegative elderly significantly reduced cellular GSH concentration when compared to seronegative control subjects. However, The GSH concentration did not significantly ($P<0.05$) vary among seropositive subjects, but were further lower than values obtained for elderly subjects. GSH concentration obtained from the study was 23.83 ± 4.86, 23.09 ± 4.22, 39.82 ± 3.07 and 29.94 ± 3.43 mg/dl in the groups NAIVE, ART, CTRL and ELD 65+ respectively.

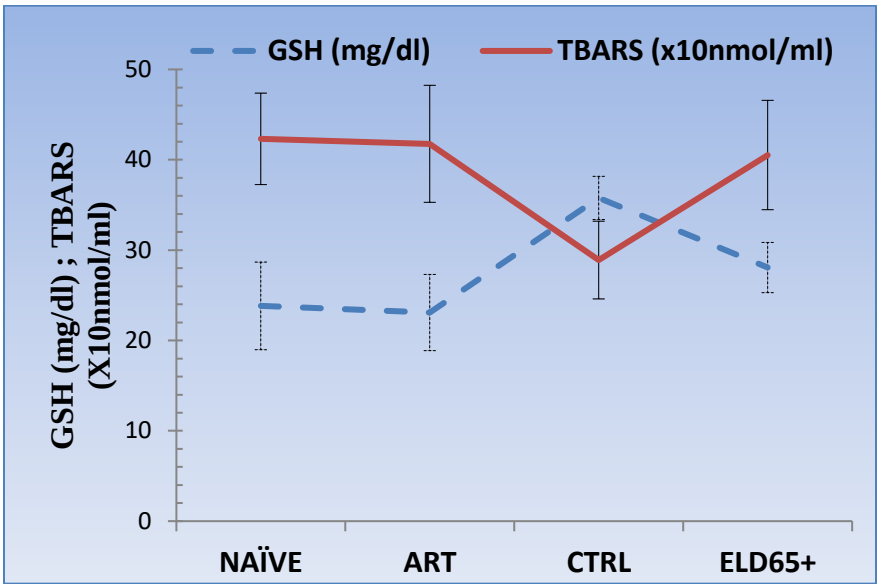

Figure 3: Glutathione (GSH-reduced) and Thiobarbituric Acid Reactive Substances (TBARS) concentrations in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART, HIV-Seronegative control subjects and HIV-Seropositive Elderly (>65) Control subjects.

3.6 Lipid peroxidation in HIV-Seropositive naïve, Seropositive on ART, Seronegative control and Seronegative Elderly Individuals

Figure 3 shows TBARS concentrations in ART-naive HIV seropositive subjects; ART treated seropositive subjects; seronegative control and elderly. The results presented indicated a significant ($P<0.05$) increase in production of thiobarbituric acid reactive substances in ART-NAÏVE subjects ($4.23 \times 10^{-9} \pm 5.06 \times 10^{-10}$) and ART ($4.18 \times 10^{-9} \pm 6.48E\text{-}10$) subjects when compared to seronegatives: CTRL ($2.89 \times 10^{-9} \pm 4.31E\text{-}10$) and ELD65+($4.05 \times 10^{-9} \pm 6.05 \times 10^{-10}$). This increases were similar to those obtained for elderly subjects ($4.05 \times 10^{-9} \pm 6.05 \times 10^{-10}$ mol/ml). Results show that malondialdehyde concentration was $4.23 \times 10^{-9} \pm 5.06 \times 10^{-10}$, $4.18 \times 10^{-9} \pm 6.48 \times 10^{-10}$, and $2.89 \times 10^{-9} \pm 4.31 \times 10^{-10}$ and $4.05 \times 10^{-9} \pm 6.05 \times 10^{-10}$ mol/ml in Naive, ART, CTRL and ELD65+ respectively.

Table 3: Mathematical model of relationship of Inflammatory, Oxidative Stress Status and Antioxidant Homeostasis in HIV-Infection And Ageing.

Inflammation, Oxidative stress and Antioxidant Parameters		Equation / Empirical Values			Procedure	Robust Minimization	Error
		Logistic dose response and sigmoid model			Levenberg Marquardt	Least Squares	
		$y = \frac{1}{1+\left\{\frac{x}{b}\right\}^{c}}$ ..... Eqn 1			r^2Coef Det	DF Adj r^2	Fit Std Err
		$y = \frac{a}{1+\exp\left\{-\frac{x-b}{c}\right\}}$ ..... Eqn 2					
x	y	a	b	c	r^2	r^2	
GSH	TBARS	42.35	38.71	9.71	0.998	0.994	0.46
CRP	ESR	54.95	21.54	9.58	0.985	0.955	3.59
GSH	CRP	41.66	3.20	2.81	0.986	0.958	1.30

Equation (1) is logistic dose response (abc), Equation (2) is Sigmoid model (abc)

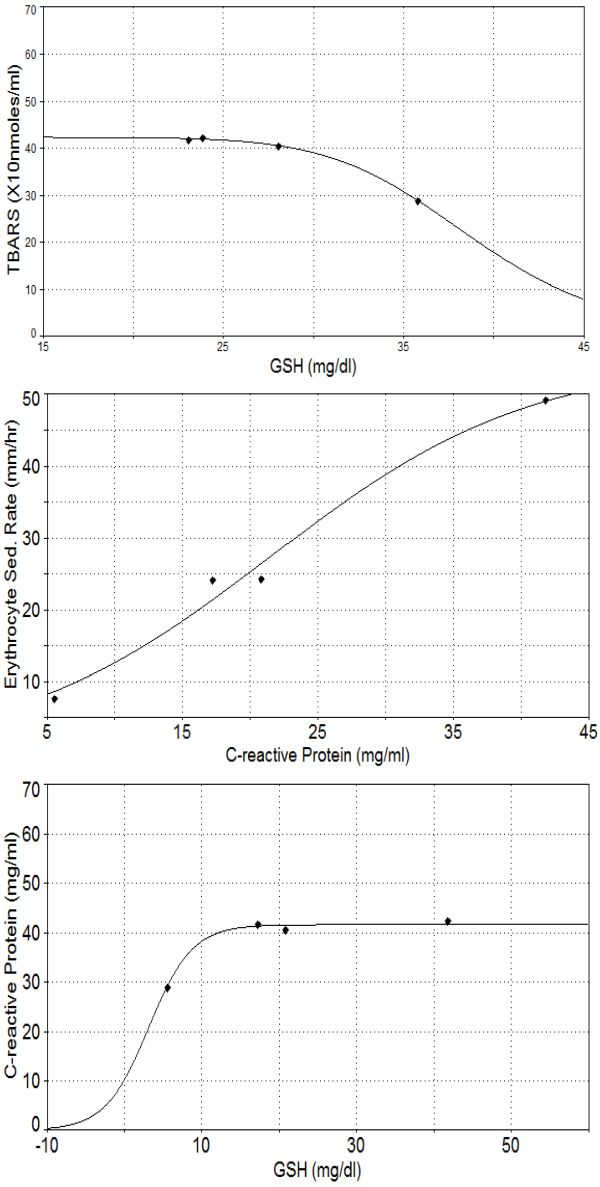

Figure 4: Logistic relationship that exists between inflammation, peroxidation and antioxidant homeostasis in HIV-disease and Ageing in humans. (A) Peroxidation and antioxidant homeostasis, (B) Inflammation parameters. (C) Inflammation and antioxidant homeostasis Plot A obeyed the Logistic Dose Response (Eqn 1) while Plots B and C obeyed the sigmoid model (Eqn 2) as derived from table 3)

4.0 DISCUSION

We determined whether Inflammatory, Oxidative Stress Status and Antioxidant Homeostasis in HIV-Infection and Ageing are Tightly Logistic in Humans. Increase in ESR was observed among the seronegative elderly and HIV-seropositive young adults in agreement with the findings of Hoffbrand and Moss 2016, Bimpong and Burthem 2017. They reported an age-dependent ESR results. Our result could be due to heightened pro-inflammatory environment that is associated with ageing and HIV disease. Increases in ESR concentration followed similar trend with the C-reactive protein concentration in our study (R^2 = 0.985). More generally, CRP is one of the many acute phase reactants that is elaborated in response to inflammation and/or tissue injury, and its rise is commensurate with

inflammatory mediators (cytokines) produced by cells actively participating in the milieu of tissue injury such as IL-6 (Markanday, 2015; Christopher *et al.,* 2016). HIV infection can be said to be inducing expression and secretion of IL-6 by monocytes and macrophages and this dysregulation is a major contributor to the pathogenesis of chronic inflammation seen in both ageing and HIV disease hence the result of the elderly is similar to those of ART (figure 2). This increase of IL-6 observed in both HIV-infection and ageing in this study may be contributing, along with other pro-inflammatory factors, to the chronic inflammation in ageing and HIV-infection.

The result of the effect of HIV disease and ageing on erythrocyte lipid peroxidation indicated that systemic oxidative stress, of which lipid peroxidation represents a major manifestation, played an important role in HIV disease (Figure 3). Lipid peroxidation was significantly higher in the erythrocytes of seropositives-naïve and ART individuals than controls. Increased erythrocyte lipid peroxidation in the seronegative elderly control (ELD65+) against seronegative younger subjects (CTRL). During HIV disease and/or in aging, there is an increased production of pro-oxidants that should have been balanced by the synthesis of antioxidants (van Mommah *et al.,* 2015; Ivanov *et al.,* 2016; Liguori *et al.,* 2018). However this delicate balance tilts in the direction of increase peroxidation as a result of diminished antioxidant concentration that favours oxidative lipid damage. Several studies corroborate this report that serum lipid peroxide levels in HIV disease and in the elderly were signifcantly higher than those in Control (wan Mohamad *et al.,* 2015; Ivanov *et al.,* 2016). HIV-1 induces oxidative stress by deregulation of oxidative stress pathways with escalation of ROS production and by inducing mitochondrial dysfunction (Ivanov *et al.,* 2016). Aging is the progressive loss of tissue and organ function over time (Flatt 2012). Several studies have documented an increase in reactive Oxygen and Nitrogen Species (RONS) in aging (Liguori et al., 2018, Ivanov *et al.,* 2016, van Mommah 2015) in keeping with increased peroxidation. The significant increase in lipid peroxidation observed in the seronegative elderly compared to our control is suggestive that HIV disease like advancement in age caused an increased oxidative damage to macromolecules like lipids (Liguori *et al.,* 2018). The exact mechanism of oxidative stress-induced ageing is still not completely elucidated, but it's been suggested that almost certainly increased ROS concentrations lead to cellular senescence, a physiological system that stops cellular proliferation in answer to damages that occur during replication.

Reduced glutathione (GSH) constitutes the first line of defense against free radicals. Due to its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Meister and Anderson, 1983). Glutathione concentration in tissues therefore runs an inverse relationship with the concentration of thiobarbituric acid reactive (Alisi et al 2011). TBARS concentration is directly proportional to/and indicative of degree of lipid peroxidation and related inversely to glutathione concentration in a dose dependent fashion that mimicked logistic dose response model abcd with $R^2 = 0.991$ (Table 3). Higher glutathione concentration indicates higher antioxidant status. The above observations meant that peroxidation in HIV-infection and ageing is tightly logistic in humans and increases or reduce strictly in a mathematical fashion that is related to antioxidant status.

Cellular responses to chemical perturbations have been shown to follow logistic models (Alisi et al 2011). The inverse association seen in glutathione and malondialdehyde concentrations are because glutathione works to protect the cell against oxidative attack and peroxidation, so if glutathione protection is overwhelmed, peroxidation increases. Reduction in serum glutathione seen in association with increased lipid peroxidation in HIV-infection and ageing indicated an antioxidant diminution resulting from increase in oxidative stress which may have resulted from chronic inflammation.

5.0 CONCLUSION

In all the parameters measured, ART subjects were similar to ELD65+ subjects suggesting immune ageing. The antioxidant parameter GSH had an inverse relationship with the inflammatory (ESR, CRP and IL-6) and oxidative stress (TBARS) parameters. This relationship was logistic and followed logistic dose response relationship and a sigmoidal association. We observed that Erythrocyte sedimentation rate, C-reactive protein, Interleukin-

6 (IL-6), Glutathione (GSH) and Malondialdehyde (TBARS) are useful parameters to assess immune ageing, and conclude that Inflammatory, Oxidative Stress Status and Antioxidant Homeostasis in HIV-Infection and Ageing are Tightly Logistic in Humans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. "Precious Nc. Alisi, Z.C. Jeremiah, Evelyn M. Eze, and Edna O. Ibegbulem designed the study, Precious Nc. Alisi performed the statistical analysis, mathematical modeling and managed the analyses of the study and literature searches. Precious Nc. Alisi wrote the protocol and the first draft of the manuscript and incorporated all corrections from co-authors. All authors read and approved the final manuscript."

CONSENT

We declare that informed consent was obtained from the subjects. There was an absolute assurance of confidentiality of the patient. Study was performed in accordance with ethical standards of the Helsinki declaration of the World Medical Association and participants gave written informed consent.

Ethical Approval

The ethical permit (FMC/OW/HREC/VOL.1- 12735) was obtained from the appropriate authority before samples were collected.

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