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Antiretroviral Therapy Immune Function Reinstallation in HIV-Infection in Sub Sahara Africa Population

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PNA, ZAJ, EME and EOI designed the study. Authors PNA and CSA performed the statistical analysis and managed the analyses of the study and literature searches. Author PNA 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.

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ABSTRACT

Aims: To access antiretroviral therapy immune function reinstallation in HIV-infection in a sub-Sahara Africa population.
Study Design: The study was carried out in HIV seronegative healthy young (20-35 years) adults (CTRL), HIV Seropositive ART-Naive (20-35 years) young adults (Naïve) and HIV Seropositive on Anti-retroviral therapy (ART) aged 20-35 years old.
Place and Duration of Study: One hundred and fifty subjects were used for the study. One hundred (100) are HIV seropositive individuals, 50 on ART and 50 ART-Naive attending the Federal Medical Centre Owerri between August and December 2020. Fifty (50) healthy younger adults of the same age range served as controls in this study.

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Methodology: EDTA and plain vacutainers were used to collect Venous blood from each participant. The following parameters were determined in the subjects: Viral load, CD4+, CD8+, CD4+/CD8+ ratio was calculated and CD57+ measured, Erythrocyte Sedimentation rate (ESR), and C-reactive protein (CRP) concentrations were also determined in subjects. The data generated were analysed by a one-way analysis of variances (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21.

Results: The result of the study showed that Naïve individuals had a mean viral load of 41.54±13.39 copies/ml (IQR12-18) while subjects on ART had a significantly ($p<0.05$) lower mean viral load value of 22.26±11.31 (IQR8-54). CD4+ count obtained was 335.46 ± 76.75, 482.80 ± 88.69 and 846.08 ± 231.47cells/mm^3 in NAIVE, ART and Control subjects respectively. CD8+ count obtained was 604.34 ± 126.09, 441.48 ± 94.42 and 376.86 ± 51.17 cells/mm^3 in NAÏVE, ART and CTRL,respectively. Our result showed that HIV infection significantly ($P<0.05$) decreased CD4+/CD8+ ratio. CD57+ counts in ART treated subjects were found to be improved by treatment. CD4+ count correlated positively with viral load in NAÏVE ($r = 0.799$) and in ART subjects ($r = 0.809$). ART-NAÏVE, ART and CTRL subjects had IFN-γ concentrations of 186.44 ± 38.67, 161.83 ± 37.34 and 133.73 ± 25.97 pg/ml respectively. 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) when compared to CTRL (7.66 ± 0.61 mm/hr). ART treatment resulted in a significant ($P<0.05$) decrease in ESR among seropositives. CRP concentration was significantly ($P<0.05$) increased in NAÏVE when compared to CTRL and subjects.

Conclusion: We conclude that Antiretroviral therapy in HIV-seropositive individuals acts to reinstall immune function and normalize fuction. Although immune function was not completely normalized, it was closer to normal than ART-naïve person whose immune function was largely compromised.

Keywords: Viral load; immune response normalization; CD4+/CD8+; immune function.

1. INTRODUCTION

The Nigeria National HIV/AIDS Indicator and Impact Survey (NAIIS). Findings provided Nigeria with an accurate national HIV prevalence measure of 1.4%. NAIIS also showed that Nigeria is able to effectively provide antiretroviral treatment. Nigeria (a sub-sahara African Nation) had the third largest infected population (2 to 3.2 million) of people living with HIV/AIDS (PLWHA) in the world after South Africa and India. Nigeria has shown steady advancement on growing access to therapy for people living with HIV, with the implementation of a test and treat policy in 2016. This measure has further accelerated referrals to treatment facilities for people who test positive for the virus. From 2010 to 2017, Nigeria almost tripled the number of people living with HIV having access to antiretroviral therapy, up from 360 000 people in 2010 to more than 1 million people in 2018. It is important that all people living with HIV get treatment and achieve viral suppression.

The immune system is a collection of cells, chemicals and processes that function to protect the body against invading microbes [1]. They, play a role in health and disease. Understanding this role is key in the pathogenesis of diseases including HIV. Depletion of the immune cells necessary to maintain immune competence eventually coincides with the onset of AIDS [1]. It is now common knowledge that inflammation causes accelerated viral replication, increase the rate of virus entry and results to immune dysfunction in HIV infection. The immune activation seen in inflammation is an important factor in the immune impairment associated with HIV/AIDS progression with consequent deranged CD4, CD8, CD4/CD8 ratio, CD57 etc.

The arrival and availability of Antiretroviral Therapy (ART) have reduced the incidence of opportunistic infections that pose threats to the existence of HIV positive individuals [2]. Antiretroviral therapy has in no small measure conferred viral suppression on people living with HIV and improved their quality of life. The optimal timing for initiating antiretroviral therapy (ART) in individuals infected with HIV-1 has not yet been resolved. The US Department of Health and Human Services and the International Antiviral Society – USA recommend starting ART as close to diagnosis as possible. A few workers did recommend initiating ART at $CD4^+$ T-cell counts of 350–500 cells/μl, but not above 500 cells/μl compared to initiating it later when $CD4^+$ T-cell counts fall below 350 cells/μl. What ever the case may be, the consensus is that ART initiation is important to prolong normal immune

function or otherwise a slide to AIDS. The aim of this work is to study the antiretroviral therapy immune function normalization and/or prolongation in HIV-Infection in Sub Sahara Africa Population.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at Federal Medical Centre Owerri in Imo State of Nigeria and is located in the South East between latitude $4^{o}45^{'}$N and $7^{o}15^{'}$N, longitude $6^{o}50$'E and $7^{o}25$'E. The Federal Medical Centre is one of the tertiary referral centres providing satisfactory medical care to HIV-infected persons in Nigeria via the heart-to-heart and sundry clinics.

2.2 Study Design

The study was carried out in three groups viz:

Group A = 50 HIV(+) subjects (age 20 to 35) who were Anti retroviral therapy -naive (NAIVE).

Group B = 50 HIV(+) subjects (age 20 to 35) on Anti retroviral therapy (ART).

Group C = 50 HIV(-) subjects (age 20 to 35) were used as controls (CTRL).

2.3 Study Population and Sample Size

This study was a cross-sectional research and was conducted prospectively among patients visiting the General out-patient department (GOPD) and the heart-to-heart clinic of the Federal Medical Centre Owerri and the populace. The minimum sample size was obtained as previously described [3] using the formula by Naing et al., 2006. The prevalence rate of seropositive in South Eastern Nigeria is 1.9% [4].

2.3.1 Selection criteria

The subjects were selected under defined criteria.

HIV seropositive subjects included in this study were generally 20-35years old. Those on Anti-retroviral therapy (ART) would have spent at least 3 months on therapy. People, herbal/complementary medicines, people on mind-altering medications, and subjects positive for HbsAg and HCV are excluded from the study. Pregnant or planning to be pregnant in the next 4 months were also excluded.

HIV seronegative: Subjects (CTRL) are generally negative for HIV, HBV and HCV. They are aged 20-35 years. People on herbal/complementary medicines or people on mind-altering medications, pregnant women were excluded and people positive for HbsAg and HCV were also excluded from the study.

2.4 Blood Sample Collection

6 mls of venous blood was drawn from the antecubital vein for haematological and biochemical analysis. About 3 mls was put into an EDTA vacutainer for determinations that require whole blood (CD4+, ESR). 3 mls was also put into an EDTA vacutainer centrifuged for 5minutes at 3000rpm to separate the plasma. The separated plasma is stored in a refrigerator for estimation of CD8+, CD57+, and CRP.

2.5 Determination of CD4+ Count

The CD 4 and CD4% count was carried using the CyFlow Counter –sysmex partec machine a fully-equipped portable/desktop flow cytometers with laser excitation in the green. It works on the principle of light scatter (due to different size or granularity of the cell) combined with fluorescence of cells after staining with monoclonal antibodies to cell surface markers tagged to fluorescent dyes. The population of interest can be identified and gated. The percentage of CD4 T cells was calculated (% of lymphocytes). Absolute CD4 count was determined directly from flow cytometer by counting CD4 T cells in a precisely determined blood volume.

2.6 Determination of CD8+ Count

The commercial Human Cluster of Differentiation 8 (CD8+) ELISA kit of Melsin Medical Co., Limited was used according to manufacturers specification. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay CD8 in Human serum, blood plasma, urine, and other biological fluids. Briefly, standard, test sample and HRP-labeled CD8 antibodies were added to microtitre wells which are Pre-coated with CD8 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 450 nm. The concentration of CD8 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.7 CD4/CD8-ratio

This was calculated. It is the ratio of T helper cells (with the surface marker CD4) to cytotoxic T cells (with the surface marker CD8). The CD4+/CD8+ ratio is a reflection of immune system health.

2.8 CD 57 count

The commercial Human Cluster of Differentiation 57 (CD57) ELISA kit of Melsin Medical Co., Limited was used. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay CD57 in Human serum, blood plasma, urine, and other biological fluids. Standard, test sample and HRP-labeled CD57 antibodies were added to microtitre wells which are Pre-coated with CD57 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 CD57 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.9 Determination of Erythrocyte Sedimentation Rate (ESR)

The measurement of erythrocyte sedimentation rate (ESR) was carried out by Modified Westergren Method [5] ESR was set up within six hours after collection of blood using the Modified Westergren Method as previously described [3].

2.10 Determination of C - Reactive Protein (CRP) Concentration

The Finecare® CRP rapid quantitative test was performed according to manufacturers specification. The Finecare® CRP rapid quantitative test is a fluorescence immunoassay used along with the Finecare FIA system for the quantitative determination of CRP in human whole blood, serum or plasma as previously described [3]. Briefly, 5µl of test plasma and QC plasma were added into the detection buffer tube. The lid of detection buffer tube was closed and the sample mixture was mixed thoroughly by shaking it about 10 times. 75 µl of sample mixture was pipetted and loaded into the sample well of the test cartridge. The quick test mode was used for testing: the timer was set to count down right after adding sample mixture into the sample well and left at room temperature for 3 minutes. Then the test cartridge was inserted onto the test cartridge holder of Finecare FIA system. The “test” button was pressed to start testing. Results were displayed on main screen.

2.11 Statistical Analysis

Data obtained from the study were analyzed by the use of one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21 software package. All results were given as Mean ± SD, plots as Mean ± SEM and values for $p < 0.05$ were considered statistically significant.

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=150) drawn from states in Nigerian (a sub-Saharan Africa population) including Abia (22), Adamawa (4), Akwa Ibom (2), Anambra (12), Bayelsa (3), Edo (7), Enugu (2), Imo (69), Kaduna (6) and River State (23). Study participants included a total number of 150 subjects and belonging to three groups of fifty subjects each: HIV-Seropositive ART-naïve individuals (NAÏVE); HIV-Seropositive individuals on Anti Retroviral Therapy (ART) and HIV-Seronegative control subjects (CTRL). The subjects by Gender included 84(56%) males and 66(44%) females. The mean ages by group are Naïve (29.52 ± 3.01 yrs), ART (30.78 ± 4.63 yrs) and seronegative control (CTRL) (26.30 ± 3.17 yrs).

3.2 Viral Load Count of HIV-Seropositive ART-naïve Individuals (NAÏVE) and HIV-Seropositive Individuals on Anti Retroviral Therapy (ART)

The result of our study (Fig. 1) showed that the HIV-Seropositive ART-naïve individuals (NAÏVE) studied had mean viral load of 41.54 ± 13.93 copies/ml with median value of 39.5 copies/ml (IQR12-86). This is significantly ($p< 0.001$) higher than the value obtained from the HIV-Seropositive individuals on Anti Retroviral Therapy (ART) with a viral load of 22.26±11.31 copies/ml with median value of 21.0 copies/ml (IQR8-54).

3.3 CD4+ in HIV-Seropositive ART-naïve Individuals, HIV-Seropositive Individual on ART and HIV-Seronegative Control Subjects

Result obtained from the study (Fig. 2) showed that CD4+ count were significantly ($P\leq0.05$) decreased by HIV infection in both Naive and ART treated subjects. ART treatment however, significantly improved CD4+ cell count in ART subjects when compared to the NAIVE. The CD4+ count obtained was 335.46 ± 76.75, 482.80 ± 88.69 and 846.08 ± 231.47cells/mm^3 in NAIVE, ART and Control subjects respectively.

Table 1. Demographic characteristics of studied sub-Saharan Africa population (Nigeria)

Distribution by State	(n)	PERCENT (%)	NAÏVE	ART	CTRL
ABIA	22	14.7	6	8	8
ADAMAWA	4	2.7	2	2	0
AKWA IBOM	2	1.3	1	0	1
ANAMBRA	12	8	4	3	5
BAYELSA	3	2	0	3	0
EDO	7	4.7	3	3	1
ENUGU	2	1.3	0	2	0
IMO	69	46	24	18	27
KADUNA	6	4	3	2	1
RIVERS	23	15.3	7	9	7
Total	150	100	50	50	50
Gender					
Male	84	56	20(40)	43(86)	21(42)
Female	66	44	30(60)	7(14)	29(58)
Age (yrs)					
Mean ± SD			29.52±3.01	30.78 ±4.63	26.30 ±3.17
Median			30	33	26.0
Mode			30	35	27
Min-Max			22-35	20-35	20-35

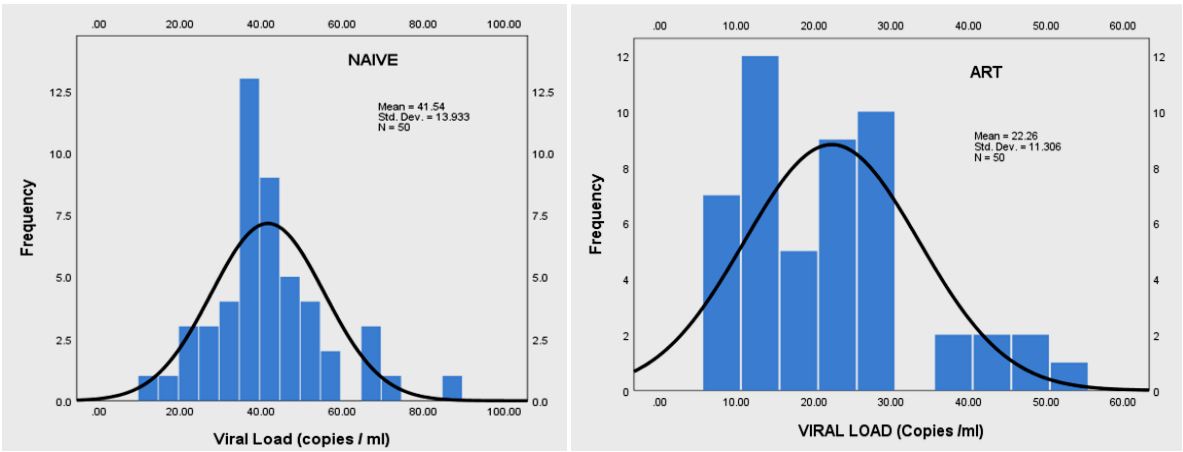

Fig. 1. Frequency Distribution of viral load count of HIV-seropositive ART-naïve individuals (NAÏVE) and HIV-seropositive individuals on Anti Retroviral Therapy (ART)

3.4 CD8+ Count in HIV-Seropositive ART-naïve Individuals, HIV-Seropositive Individual on ART and HIV-Seronegative Control Subjects

The CD8+ count results (Fig. 2) showed that counts were generally increased across seropositive NAÏVE subjects and ART treated subjects. CD8+ count in NAIVE were significantly ($P<0.05$) higher when compared to treated subjects. Further pairwise comparison showed that ART treated subjects showed improved CD8+ count and were significantly ($P<0.05$) higher than NAÏVE. In the groups NAÏVE, ART and CTRL, CD8+ count was 604.34 ± 126.09, 441.48 ± 94.42 and 376.86 ± 51.17 cells/mm^3 respectively.

3.5 CD4+/CD8+-ratio in HIV-Seropositive ART-naïve Individuals, HIV-Seropositive Individual on ART and HIV-Seronegative Control Subjects

Further analysis of CD4+/CD8+ ratio (Fig. 2) showed that HIV infection significantly ($P<0.05$) decreased CD4+/CD8+ ratio. However, ART treatment significantly ($P<0.05$) increased CD4+/CD8+ ratio in treated seropositive subjects. Comparison of the ratios showed that there were significantly different CD4+/CD8+ ratio across the different subject groups. CD4+/CD8+ ratio was 0.58 ± 0.16, 1.14 ± 0.31, and 2.29 ± 0.69 among the subjects, NAÏVE, ART and CTRL respectively. The CD4+/CD8+ ratio obtained in the study varied in the order Control > ART > naïve.

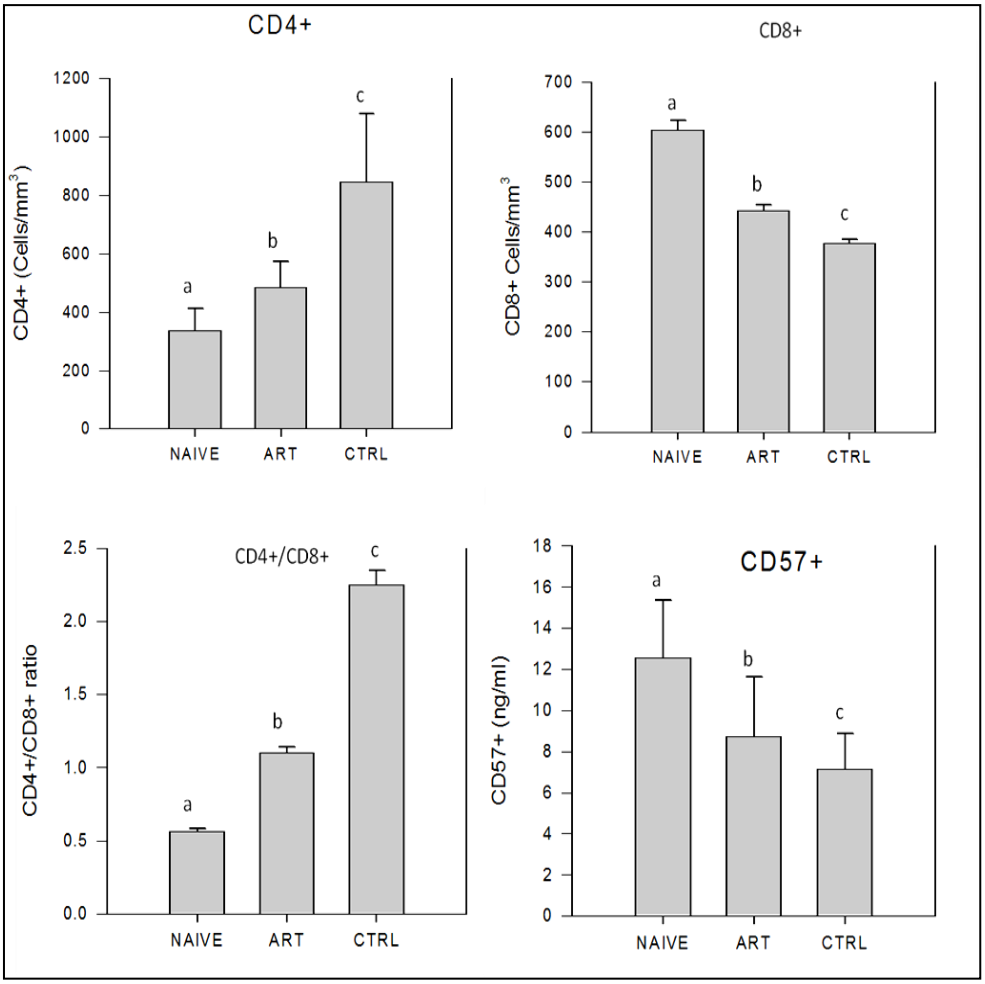

Fig. 2. CD4+ Count; CD8+ Count; CD4+/CD8+-ratio and CD57+ Concentration in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART and HIV-Seronegative control subjects

(Values are plotted as Mean ± SEM)

3.6 CD57+ in HIV-Seropositive ART-naïve Individuals, HIV-Seropositive Individual on ART and HIV-Seronegative Control Subjects

Result of CD57+ assay (Fig. 2) showed that count across the subject groups were significantly (P<0.05) different. CD57+ count were shown to be significantly (P<0.05) increased in seropositive naive subjects and ART treated seropositive subjects when compared to seronegative control subjects. However, counts in ART treated subjects were found to be improved by treatment and were closer to counts obtained for seronegative control. The CD57+ count was 12.53 ± 2.81, 8.72 ± 2.89 and 7.16 ± 1.73 pg/ml among the subjects, NAIVE, ART and CTRL respectively.

3.7 Correlation of CD4+ Count with Viral Load in HIV-Seropositive ART-naïve Individuals (NAÏVE); HIV-Seropositive Individuals on Anti Retroviral Therapy (ART) and HIV-Seronegative Control (CTRL) Subjects

Result of our study (Fig. 3) showed that viral load in seropositive subjects inversely related to the clusters of differentiation (CD4+) count. The inverse relationship was true in all seropositive subjects including ART-NAÏVE and ART subjects. CD4+ count was significantly (P<0.05) higher in ART subjects with lower viral count than the ART-NAÏVE sublects with higher viral load count. CD4+ count correlated positively (r = 0.799) in NAÏVE and also positively (r = 0.809) in ART subjects.

3.8 Erythrocyte Sedimentation Rate (ESR) in HIV-Seropositive ART-naïve Individuals (NAÏVE); HIV-Seropositive Individuals on Anti Retroviral Therapy (ART) and HIV-Seronegative Control (CTRL) Subjects

The r Result of ESR estimation (Fig. 4) in HIV seropositive and seronegative individuals showed that ESR was significantly (P<0.05) elevated in HIV seropositive subjects (NAÏVE and ART)(49.16 ± 5.49 mm/hr and 24.12 ±2.88 mm/hr) when compared to CTRL (7.66 ± 0.61 mm/hr). ART treatment resulted in a significant (P<0.05) decrease in ESR among seropositives. ESR in the NAÏVE subjects had a maximum value of 150 mm/hr, median value of 34mm/hr, minimum value of 12.0 mm/hr and a range 12-150 mm/hr. Similarly, ART had a maximum value of 95 mm/hr, median value of 15.5 mm/hr, minimum value of 3.0 mm/hr i.e. 3-95 mm/hr. CTRL had a maximum value of 20 mm/hr, median value of 7 mm/hr, minimum value of 1 mm/hr and a range of 1-20 mm/hr. In the groups NAÏVE, ART, and CTRL, ESR values were49.16 ±5.49, 24.12 ±2.88, and 7.66 ± 0.61 respectively.

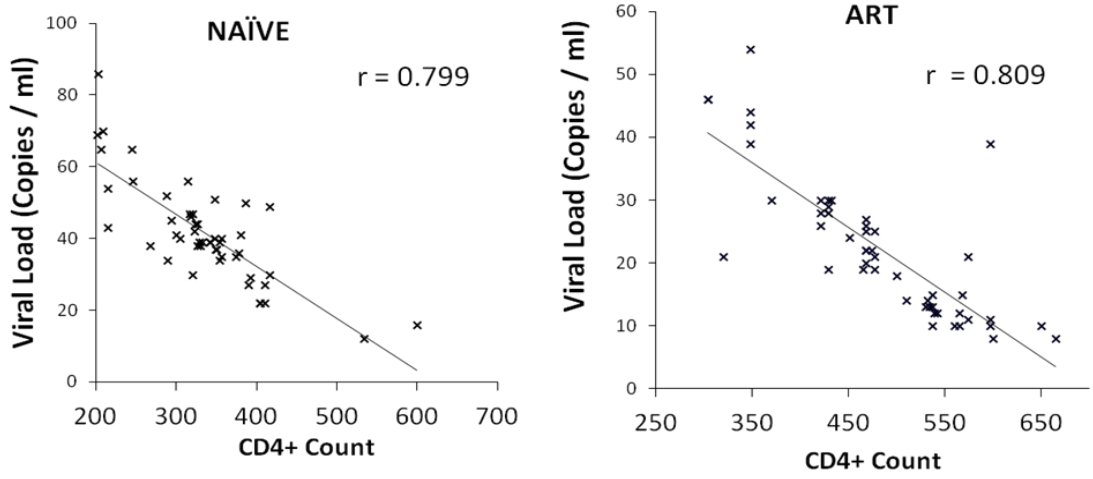

Fig. 3. Correlation of CD4+ Count with Viral Load in HIV-Seropositive ART-naïve individuals (r = 0.799) and HIV-Seropositive individual on ART (r = 0.809)

3.9 C-reactive protein (CRP) Concentration in HIV-Seropositive ART-naïve Individuals (NAÏVE); HIV-Seropositive Individuals on Anti Retroviral Therapy (ART) and HIV-Seronegative Control (CTRL) Subjects

Result (Fig. 4B) showed that the CRP concentration in NAIVE was 41.8 ± 27.6mg/l; ART was 17.2 ± 11.5 mg/l and CTRL 5.5± 3.5mg/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) and ART (17.2 ± 11.5) subjects. The treatment with ART resulted in a significant (P<0.05) decrease in CRP among seropositives. CRP concentration in the NAÏVE subjects had a maximum value of 115 mg/l, median value of 33.6 mg/l, minimum value of 12.3 mg/l and a range 12.3-115 mg/l. ART had a maximum value of 56.3 mg/l, median value of 14.5 mg/l, minimum value of 2.0 mg/l and a range of 2-56.3 mg/l. On the other hand CTRLhad a maximum value of 17.8 mg/l, median value of 4.4 mg/l, minimum value of 1.5 mg/l and a range of 1.5-17.8 mg/l.

3.10 Interferon gamma (IFN-γ) Concentrations in HIV-Seropositive Naïve, Seropositive on ART and Seronegative Control (CTRL) Individuals

The result of serum cytokine, Interferon gamma (IFN-γ) concentration (Fig. 5) in HIV seropositive and seronegative individuals showed that IFN-γ concentration was significantly (P<0.05) elevated in HIV seropositive subjects (NAÏVE and ART) compared to CTRL. NAÏVE, ART and CTRL had IFN-γ concentrations of 186.44 ± 38.67, 161.83 ± 37.34 and 133.73 ± 25.97 pg/ml respectively.

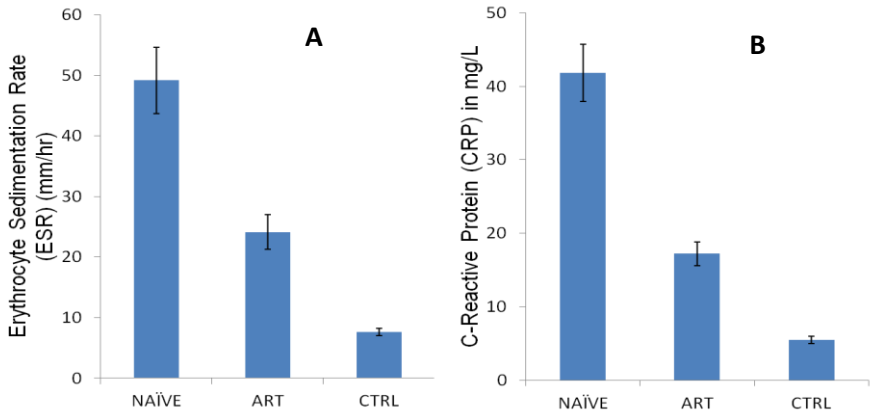

Fig. 4. Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP) Concentration in HIV-Seropositive ART-naïve individuals; HIV-Seropositive individual on ART and Seronegative control subjects (CRTL)

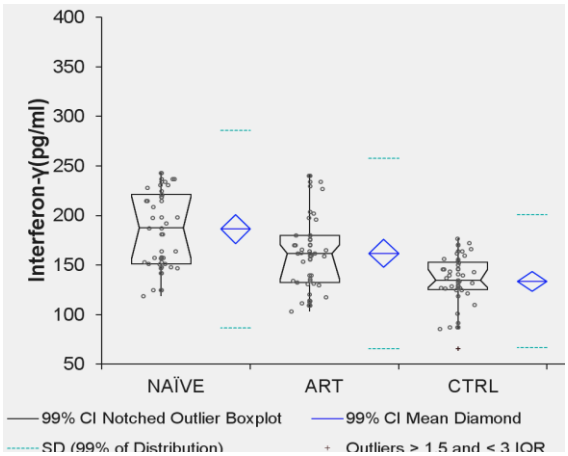

Fig. 5. Interferon-γ Concentrations in HIV-Seropositive ART-naïve individuals, HIV-Seropositive individual on ART and HIV-Seronegative control subjects (CTRL)

4. DISCUSSION

In the present study result obtained showed that CD4+ count and CD4% (Fig. 2) were decreased by HIV infection in both naive and ART-treated subjects. ART treatment, however, improved CD4+ cell count in treated seropositive subjects when compared to the naïve subject. Many studies just like ours have documented a decrease in CD4 among HIV infected individuals [6,7,8,9,10]. The binding of HIV with the target cell is through a receptor mechanism. The gp120 of the virus envelope will specifically bind with the CD4 molecule and a co-receptor (either CCR% or CXCR4) of the host cell on the surface of the target cells. Once bound, one of several co-receptors is necessary for the process of fusion and for the viral particle to disgorge its contents, i.e., two copies of the viral RNA. Thus CD4 acts as a receptor for the virus. The CD4 molecules are there on the surface of T-helper cells and therefore helper cells receive the highest attack of HIV. This brings about a general immune activation which results in unremitting damage of CD4 T lymphocytes by HIV.

CD4+ Th cells play an important role in establishing and maximizing the immune response. These cells have no cytotoxic or phagocytic activity, and cannot directly kill infected cells or clear pathogens. However, they "mediate" the immune response by directing other cells to perform these tasks and regulate the type of immune response that develops.

CD8+ T-cells are a critical component of the cellular immune response and they play a role in the control of viral infection [11]. The CD8+ count results (Fig. 2) showed that counts were generally increased across seropositive naïve subjects and ART-treated subjects. CD8+ count in naïve subjects was higher when compared to treated subjects. Further pairwise comparison showed that ART-treated subjects showed improved CD8+ count. Perdomo et al., 2019 documented that the total pool of circulating CD8+ T-cells is persistently increased in untreated HIV-infected patients compared with seronegative individuals [12], with a higher frequency of memory subsets and reduction of naïve cells [13,14]. IFN-γ secretion by CD8+ T cells exposed to HIV antigens is a widely used metric for identifying antigen-specific responses as seen in our result (Fig. 5) and does not correlate with HIV control [11]. The reason for our observed increase in CD8+ among the HIV seropositive individuals is because CD8+ T-cells can recognize infected cells through an MHC-1 dependent process and can lyse cells harbouring HIV by the secretion of perforin and granzymes. This action of CD8+ T-cells are critical in the natural and cART-induced control of viral replication; however, CD8+ T-cells are highly affected by the persistent immune activation and exhaustion state driven by the increased antigenic and inflammatory burden during HIV infection, inducing phenotypic and functional alterations, and hampering their antiviral response. Therefore an effective CD8+ T-cell response is required to control viral replication and the reservoir size.

CD4+/CD8+ ratio was decreased by HIV infection from our result (Fig. 2). However, ART treatment increased the CD4+/CD8+ ratio in treated (ART) seropositive subjects. Comparison of the ratios showed that there was a significantly difference of CD4+/CD8+ ratio across the different subject groups with that of CTRL being the highest. Studies have reported an increase in the ratio of CD4 to CD8 T cells to be greater than 1, as is expected in healthy adult individuals with no obvious infections [15]. A CD4/CD8 ratio less than 1 as seen in our NAÏVE is associated with biomarkers of activation and inflammation and is predictive of HIV and non-AIDS-related morbidity and mortality [16]. The increase in CD4/CD8 ratio in HIV treatment as observed among our ART is an important tool in the measurement of the success of treatment strategies that limit persistent inflammation. The assumption is that with the initiation of ART CD4, CD8 and CD4/CD8 ratio should return to normal but Mutoh et al 2018 reported an incomplete recovery of CD4 count, CD4%, and CD4/CD8 ratio to the levels seen in healthy individuals even after long-term successful cART in patients with a suppressed VL and this agreed with our findings in the present study in which there was an incomplete revitalization. This was similarly supported by reports of [17,18,19] that stated that initiating ART during acute HIV infection, at higher baseline CD4 cell count and CD4/CD8 ratios were associated with shorter time to CD4/CD8 ratio "normalization" and Suppressed viremia during Long-term Antiretroviral Therapy.

CD57 is first characterized as a Natural Killer cell marker and so has been most widely explored as a marker of replicative senescence on T cells [20]. $CD57^+$ cells have short telomeres, low telomerase activity, low expression of cell-cycle associated genes and limited proliferative

capacity [21,22]. In the present study (Fig. 2), CD57+ count increased in seropositive NAÏVE and ART compared to the controls showing increased replicative senescence among the HIV-Naïve which is reduced by antiretroviral therapy as counts in seropositive subjects were found to be reduced by treatment towards counts obtained for seronegative control. HIV also causes several defects in the immune system although these abnormalities improve during suppressive ART but have been reported to fail to normalize in chronically HIV-infected individuals as shown by our result. However, we did not separate the chronically HIV-infected subjects which has a potential for continuous antigenic stimulation of CD8 T-cells.

5. CONCLUSION

Administration of ART to HIV-Seropositive individual in this study improve immune function but did not completely bring about normalization of immune function. The values of CD4+/CD8+ ratio were move closer to that of control subjects with more robust immunological reconstitution.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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

Ethical approval (FMC/OW/HREC/VOL.1- 12735) was sought and permit was obtained from the relevant authorities before samples collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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