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Effects of Anti-Retroviral Therapy on Some Haematological Indices of HIV Sero-Positive Individuals in Rivers State, Nigeria

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Abstract
This study was carried out to investigate the effect of antiretroviral therapy on some Haematological indices of HIV seropositive individuals in Rivers State, Nigeria. A total of 300 HIV subjects, aged 20-70 years, were recruited. They were classified into three groups; Group 1 was made up of Human Immunodeficiency Virus (HIV) sero-Positive individuals on highly antiretroviral therapy as the test subjects; Group 2 was HIV sero-Positive individual but not on highly active antiretroviral therapy as control 1; Group 3 was HIV sero-negative individuals as control 2. Ethical approval for the study and informed consent from the participants were obtained accordingly. HIV infections on already confirmed cases were further confirmed using national algorithm including determine, unigold and statpak tiebreaker tests, after pre-test counselling. The results of the haematological parameters in the seropositive patients was significantly higher (P<0.005) in HB, WBC, PCV, platelet, lymphocyte, neutrophil and CD4, compared to the normal. Also, there was significant increase (P<0.005) in HB, WBC, PCV, Platelet, Lymphocyte, Neutrophil, and CD4 of the test subject compared to control I and control II. The haematological parameters of HIV patients on Highly Active Antiretroviral Therapy (HAART) was significantly different (P<0.005) in relation to age, between sexes, and compared to control I and control II. In terms of duration of HAART, while there is significant difference in the haematological parameters of HIV positive patient who have been on it for over a year compared to control (p<0.05), there is no significant difference (p<0.05) for those who have been on HAART for 1 year and below. This study demonstrated the early findings that HAART has differential impact on males compared to women of different ages that become significant only after a year of therapy.

Keywords: CD4 cell counts, Haematological indices, Highly Active Antiretroviral therapy, HIV sero-negative, HIV sero-Positive

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Introduction

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

Antiretroviral treatment reduces both the mortality and the morbidity due to HIV infection (Pallela *et al.*, 1998; Ebonyi *et al*., 2017). HIV infects primarily the vital cells in the human immune system such as helper T Cells (to be specific, CD_4^+ T cells), macrophages, and dendritic cell.

Currently, more than 25 antiretroviral drugs are available to treat HIV) infection. A great majority of them target the HIV-1 reverse transcriptase (RT) including zidovudine, didanosine, zalcilahinc, stavudine, lamivudine, abacavir, emtricitahine and tenofovir (nucleoside and nucleotide RT inhibitors), nevirapine, delavirdine, efavirenz and etravirine (non-nucleoside RT inhibitors), and the viral protease inhibitors: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atanavir, fosamprenavir, tipranavir and darunavir.

Antiretroviral therapy (ART) has been reported to improve the health and prolong the lives of most sero-positive patients, as compared to other therapies (Zerihun *et al*., 2019). HAART is observed to alter the course of HIV disease dramatically with decreased mortality and morbidity since its introduction in the 1990s.

There are some documented reports on the cytopenic effect of antiretroviral drugs on patients (Gebremedhin and Haye, 2019). For instance, lamivudine and zidovidine combination as well as stavudine have been reported to cause neutropoenia, anaemia, thrombocytopaenia and even transient rise in liver enzymes and serum amylase (EMA, 2015; Zerihun*et al*., 2019). This work was carried out to evaluate the extent of the effect of HAART on the haematological indices of HIV sero-positive individuals in Rivers State, Nigeria. The outcome was expected to help in the determination of the most effective and the safest drug combination for HAART. In addition, it will help to determine if haematological response can be employed as a veritable tool for monitoring patients on treatment in cases where CD4 count cannot be done.

Materials and Method

Study Area

This study took place in the University of Port Harcourt Teaching Hospital, Choba, Port Harcourt, Rivers State, Nigeria from 26th July, 2016 – 23rd May, 2017. University of Port Harcourt Teaching Hospital serves as both a primary contact hospital and a regional referral hospital. Currently, the ART clinic provides HIV related services for both adults and children.

Port Harcourt, the capital of Rivers State, is located in South-eastern Nigeria (07^o 3’ E, 04^o 51’N, and 10m altitude above sea level) in the humid forest zone of the Niger Delta region, Nigeria. It is densely populated and home to multinational oil and gas companies and as such witnesses the influx of people in search of better living.

Port Harcourt features a tropical wet climate with lengthy and heavy rainy seasons and very short dry seasons. Temperatures throughout the year in the city are relatively constant, showing little variation throughout the course of the year. Average temperatures are typically

between 21^{o}C-31^{o}C in the city (https://weatherspark.com/y/54960/Average-Weather-in-Port-Harcourt-Nigeria-Year-Round).

Study Population

A total of three hundred (300) subjects within the age range of 20-70 years were recruited for this study: two hundred (200) were undergoing treatment for HIV infection, and 100 healthy individuals. They were classified into three groups: (1) HIV sero-positive individuals on ART (2) Control 1 – HIV sero-positive individual but not on ART (3) Control 2 – HIV negative individual. The first batch was 100 in number, that is, HIV sero-positive on ART for the minimum of seven months, and the second batch was 100 in number for HIV sero-positive individuals not on ART. The third batch was 100 that is HIV negative.

Advocacy, Mobilization and Pre-Survey Contacts

A letter of introduction from the Head,Department of Biomedical Sciences, University of PortHarcourtand a detailed proposal stating the purpose of researchwere submitted to the Chief Medical Directorand the ethical committee of University of Port Harcourt Teaching Hospital, and approval was obtained.

Scheduled meetings were held with the study subjects and writteninformed consent was gotten from them with structured questionnaires distributed with the help of field assistants.

Selection Criteria

Inclusion Criteria: HIV sero-positive individuals on ART for 7 months and above**,** HIV sero-positive individuals on ART within the age bracket of 20-70 years**,** HIV sero-positive individuals on ART on a given consent**,** HIV sero-positive individuals without ART on a given consent and HIV sero-negative individuals.

Exclusion Criteria: Subjects with chronic disease aside HIV, e.g. liver disease, renal disease**,** HIV sero-positive individuals outside the age bracket.

Parameters Studied

The parameters evaluated in this study include: Haemoglobin (Hb), White Blood Cell (WBC), Packed Cell Volume (PCV), Platelet, lymphocyte, and Neutrophil (Neu).

Study Design

Baseline blood samples were collected from HIV sero-positive patients on anti-retroviral drugs (Group 1), HIV sero-positive patients without antiretroviral drugs (Group II), and HIV negative participants (Group III). Group I participant continued with their therapy for another 3 months when blood samples were collected for analysis. Samples were collected between 9 am-12 pm to reduce variability. Samples were centrifuged and stored at -20^{o}C. Estimation of haematological parameters was carried out using appropriate reagents and equipment.

Laboratory Procedures

Blood Sample Collection

Five millilitre syringe was used to draw blood from the anti-cubital vein of each subject. The blood was dispensed into a tube containing ethylenediamine tetra-acetic acid (EDTA) and was used immediately for haematological parameters.

Haematological Study

$CD4^+$ Count

This was done using $CD4^+$Dynal bead techniques which uses the principle of immune-magnetic cell isolation using Dynal bead at T4-T8 Quant, enables rapid quantification of T4 and T8 lymphocytes directly from samples of EDTA and anti-coagulated blood. This is based on isolation technique of depletion of Monocytes, isolation of CD4+ cells and $CD4^+$T cells counting.

Packed Cell Volume Determination

Blood sample was drawn into capillary tube and sealed. It was placed in a haematocrit centrifuge. Centrifugation of blood was carried out at approximately 12000 g for 5 minutes and PCV was determined by a haematocrit reader.

Haemoglobin Estimation

This was carried out using the method of Van Kampen and Zilistra, (1961).

White Blood Cell Count

This was carried out using the method of Baker.

HIV Test

This was done using the method of CHEMBIO by Essex et al. 1983

Statistical Analysis

Results were analysed using Statistical Package for Social Sciences (SPSS) version 15. The data were expressed using descriptive statistics and Analysis of Variance (ANOVA). Multiple comparisons for the parameter were done using Post Hoc Turkey (HSD) to test for the level of significance between means.

Values were expressed as mean ± Standard Deviation (m ± SD) and values above the 95% confidence limit ($P<0.05$) were considered statistically significant.

Results

The values for the haematological parameters of normal subjects are shown in Table 1 below:

Table 1. Haematological Parameters of Normal Subjects

GROUP	Hb	WBC	PCV	PLAT	LYMPH	NEU
Normalsubjects	12.5420± 1.96171	7.8460± 2.37404	39.7100± 7.10142	258.06± 52.77182	48.8600± 5.71728	51.1400± 5.71728

F=25.703; *p*=0.000

The values for the haematological parameters of HIV positive subjects are shown in Table 2 below:

Table 2. Haematological Parameters of HIV Positive Subjects

Group	Hb	WBC	PCV	PLAT	LYMPH	NEU	CD4
HIV^+	10.67±1.93	4.76±1.19	31.67±6.19	236.70±82.05	35.15± 7.42	49.87±9.17	401.50±133.64

F=1.200; *p*=0.000

There is a statistically significant difference in the haematological parameters of HIV positive subjects on HAART and that of subjects not on HAART (F=1.255, P=0.020). This showed that HAART treatment from these studies has the capability of reducing the incidence of anemia, lymphopoenia and thrombocytopenia which are associated with HIV/AIDS disease progression and death in infected patients. It also revealed higher CD4 count of HIV patients on HAART relative to HIV subjects not on HAART, as presented in Table 3 below:

Table 3. Haematological Parameters of HIV Positive Subjects on HAART and those not on HAART

GROUP	Hb	WBC	PCV	PLAT	LYMPH	NEU	CD4
HIV POSITIVE SUBJECTS ON HAART	10.6707± 1.92742	4.7677± 1.18931	31.6737± 6.19648	236.70± 82.04683	35.1545± 7.41908	49.8697± 9.16847	3.2133± 137.90673
HIV POSITIVE SUBJECT NOT ON HAART	11.5230± 7.37739	4.5830± 1.30004	33.7400± 6.70838	216.07± 79.65535	42.6700± 11.57152	43.8400± 14.02935	313.112± 140.85848

F=1.255, *p*=0.020

There is a statistically significant difference in the haematological parameters of HIV positive patients on HAART based on age (<26 years, F=1.009, P=6.47; 26-35 years, F=0.000, P=0.000; 36-45 years, F=0.000, P=0.000; 46 years and above, F=0.000, P=0.000), as presented in Table 4 below:

Table 4. Haematological Parameters of HIV on HAART in Relation to Age

GROUP	Age	Hb	WBC	PCV	PLAT	LYMPH	NEU	CD4
HIV POSITIVE ON HAART	<26 years F=1.009 P=6.47	11.231± 1.52072	4.2700±. 90314	29.8400±10.42542	230.302± 64.45507	36.990±7 .88775	48.6556±.8.1 3682	394.50±186.24446
	26-35 years F=0.000 P=0.000	10.940± 2.12718	4.6400±. 66030	31.8200±9 .24792	232.102±. 120.84099	30.4800± 6.24585	57.5000±6.88 234	337.252±122.45645
	36-45 years F=0.000 P=0.000	11.220± 1.58030	4.8400±. 87838	33.6600±4 .74089	183.402±2 4.07488	33.3900± 6.68289	55.370 00±7.5 1488	300.252±21709656
	46 years and above	11.081± 2.04197	5.6818± 1.39844	32.5636±4 .78524	239.912±6 1.03188	34.7132± 6.49905	50.625 5±1.00 096	299.402±173.83987

F=0.000; *p*=0.000

There is a statistically significant difference in the haematological parameters of HIV positive patients on HAART based on sex (<26 years, F=1.009, P=6.47; 26-35 years, F=0.000, P=0.000; 36-45 years, F=0.000, P=0.000; 46 years and above, F=0.000, P=0.000). Details are as shown in Table 5 below:

Table 5. Haematological Parameters of HIV on HAART in Relation to Sex

GROUP	Sex	Hb	WBC	PCV	PLAT	LYMPH	NEU	CD4
HIV POSITIVE ON HAART F=0.000 P=0.000	Male	11.1800± 1.59917	4.4900±. 82523	33.8400±4. 64284	197.102± 44.44835	29.9000±6.3 1981	58.6111±5. 42297	292.752±1 64.14095
	Female	10.9833± 2.08175	4.6667±. 78655	30.9500±1 0.63781	268.002±.14 8.84623	37.6833±4.6 1277	49.4333±7. 47280	243.25226 ±9.76456

There is a statistically significant difference in the haematological parameters of HIV positive patients on HAART based on duration of therapy (0-1 year, F=1.126, P=0.601; 2-5 years, F=0.000, P=0.000; 5-10 years, F=0.000, P=0.000; 10 years and above, F=0.037, P=0.024), as shown in Table 6 below:

Table 6. Haematological Parameters of HIV on HAART in Relation to duration of Therapy

GROUP	Duration of Therapy	Hb	WBC	PCV	PLAT	LYMPH	NEU	CD4
HIV POSITIVE ON HAART	0-1 year F=1.126 P=0.601	10.7700±1.52072	4.89±1.12393	32.1400±5.19897	253.502±76.67283	35.6100±6.09726	48.4±5.24951	271.1672±150.81888
	2-5 years F=0.000 P=0.000	11.3571±2.20216	4.61±1.00598	32.3714±10.52310	258.002±139.22524	35.6143±4.85847	51.7500±7.79352	287.672±200.64479
	5-10 years F=0.000 P=0.000	11.1200±1.31415	4.980±.87838	33.6600±4.74089	166.602±9.78775	39.5800±3.21668	52.2200±4.71826	254.202±271.41150
	10 years and above F=0.037 P=0.024	8.23±2.88675	4.50±1.60935	24.7000±8.66025	350.672±298.27225	34.8333±5.61278	46.6000±13.71787	239.672±170.61164

Discussion

The results of this study showed significant difference ($p<0.005$) between the values of haematological parameters in HIV positive on HAART and that of HIV positive without HAART. This may be probably due to the reduction in viral load, decreased destruction of mature hematopoietic cells of multiple lineage and an improvement in the erythropoietic response (Ellaurie& Rubinstein, 1991; Kwame et al., 2018; Zerihun et al., 2019).

The results of this study also buttressed the fact that patients should make use of antiretroviral drugs, even when there are bearable side effects associated with the use of the drugs that doesn't warrant the use of an alternative (Iornon *et al*., 2008).

The results showed that there was significant difference ($p<0.005$) between the haematological parameters of HIV positive and the normal subject. Despite some side effects that were reported to be associated with the use of HAART, the results of this study reemphasized and encouraged the use of HAART.

PCV value was reduced in both HIV positive subject on HAART and those not on HAART to levels that should be considered as mild anaemia respectively. This confirmed reported effects of HIV/AIDS on hematopoietic parameters and blood cells (Blockman, 1991; Odunukwe et al., 2005; Ebonyi et al., 2017). But with HAART, the value of PCV increases over those who were not on HAART. This showed that HAART therapy has ability to promote blood cells production.

From the study, the age distribution of the participants used for the study, with the members of the three groups fell within the age distribution of 20-60 years of age. Similarly, the majority of the population were within the age range of 20-60 years of age.

The reported reduction in CD4+ count in this study was consistent with the established diagnosis of AIDs (James *et al*; 1998). This observed reduction was not less than 200 cells mm^{-3}. But during the HAART, there was an increase in the CD4+ count which was consistent with previously. reported outcome (Wei *et al.,* 1995; Chukwurah *et al.,* 2007)).

The reported significant increase observed in WBC count and an insignificant increase in CD4+ during this study was an indication of the ability of HAART to boost the immune system and reduce the risk of an opportunistic infection (Baker *et al.,* 2007; Umar et al., 2007). Although the study of Mohri suggested that adult immune system might not have the capacity for rapid CD4+ T cell regeneration, but there were increase in the lymphoid subset in the blood (Mohri *et al.,* 1998).

Several authors have reported anemia, neutropenia, thrombocytopenia, eosinophilia, etc as the adverse effects of HAART on some haematological parameters and, correlation between total lymphocytes count and CD4+ cells count was carried out. The results obtained in this study showed a positive capability of reducing the incidence of anaemia, lymphopoenia, thrombocytopenia which are associated with HIV/AIDS disease progression, and death in infected patients. It also revealed that the CD4 count of HIV patients on HAART is higher than that of HIV subject not on HAART (Balakrishnan et al., 2010; Ifeanyichukwu and Bright, 2016).

Conclusion

In conclusion, the results showed the ability of the drugs to boost the immune system which seems to overide the reported side effects and encouraged the management of HIV/AIDS patients onHAART despite the side effects. The drug's effectiveness will become significant if proper management start at the early stage under qualified personnel.

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