ORIGINAL RESEARCH ARTICLE

EFFECT OF COMBINATION ANTIRETROVIRAL THERAPY (cART) ON HAEMATOLOGICAL VARIABLES AMONG PEOPLE LIVING WITH HIV INFECTION IN GOMBE, GOMBE STATE, NORTH EAST NIGERIA

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ABSTRACT

Background:
Human immunodeficiency viral infection is known to cause haematological abnormalities and coagulopathies by various mechanisms, especially during its late course. Administration of combination antiretroviral therapy (cART) could positively or negatively affect these haematological parameters. Thus, determination of the effect of cART on haematological variables is required early for initiation and monitoring of the disease.

Aim and Objectives:
The aim of the study was to investigate the effect of cART on haematological variables among people living with HIV infection (PLHIV) in Gombe, Gombe state, Nigeria.

Materials and Methods:
A case control study design was conducted among 180 participants, 120 were PLHIV on cART and 60 treatment naïve controls. Blood samples were collected for CD4+ cell count, viral load and complete blood count (CBC) after obtaining informed consent; and a questionnaire was used to collect sociodemographic and clinical data.

Results:
People living with HIV infection on cART had significantly higher ($p < 0.05$) Hb (13.01 ± 1.9 vs. 11.2± 2.9g/dl), MCH (33.7 ± 5.0 vs. 28.7 ± 7.5pg), MCV (94.0 ± 11.1 vs. 81.4 ± 7.5fl), MCHC (35.6 ± 1.9 vs. 33.9 ± 3.6g/dl), and platelets (264.0 ± 106.0 vs. 245.6 ± 108.8 $x10^9$/L) than treatment naïve individuals. There was no statistically significant difference in WBC count between the two groups, neither was there a significant difference among the groups based on type of cART regimen.

Conclusion:
This study confirms that, cART has positive impact on some haematological variables; thus, has the ability to reduce viral load and boost immune system of PLHIV. The different cART regimens had similar effects on the changes in the haematological variables

Keywords:
HIV, combination antiretroviral therapy, haematological variable

INTRODUCTION

Haematological variables such as haemoglobin, packed cell volume (PCV), total white blood cells (WBC), myeloid cells, lymphoid cells, thrombocytes, and indeed all lineage of blood cells are the

most common parameters affected among people living with HIV infection (PLHIV), especially during the advanced stage of the diseases.[1] Anaemia has been reported to be the most common in PLHIV especially among those with advanced disease.[2] It is independently associated with increased morbidity and mortality risk [3], and remains the most common problem among treatment naïve PLHIV in Sub-Saharan Africa especially, in Nigeria, [4] which is usually normocytic-hypochromic and it is associated with a low reticulocytes count.[5] The causes of anaemia are numerous and its pathophysiology involve certain mechanism, such as, decreased red blood cell (RBC) production by direct effect of human immunodeficiency virus (HIV) infection, effect of opportunistic infection, decreased production of erythropoietin, increased RBC destruction as a result of autoimmune antibodies and nutritional deficiencies such as vitamins and iron deficiencies more especially in developing countries, such as Nigeria.[6] Leucopenia, especially neutropenia is frequently observed in advanced stages of HIV infection after development of acquired immunodeficiency syndrome (AIDS), and has been associated with certain types of antiretroviral medication used to treat HIV infection.[7] Thrombocytopenia characterized by low platelets count below $150x10^9$/l also frequently occur in people living with HIV infection.[8] The pathogenesis of thrombocytopenia in HIV infection is not fully understood but probable mechanisms reported are destruction of thrombocytes, either caused by non-specific deposition of circulating immune complex on the circulating platelets or presence of specific anti-thrombocytes antibodies, as well as direct infection of megakaryocyte by HIV which probably lead to decrease in thrombocytes production.[9]

Haematological variables (Packed cell volume, total white blood cell count, haemoglobin, platelet count, differential white blood cells count and total red blood cells count) have not been studied among PLHIV in Gombe State, Nigeria. This study is aimed at providing valuable information for effective diagnosis, therapeutic and prophylactic procedures, as well as understanding of the aetiology and pathogenesis of effect of cART on haematological variables.

MATERIAL AND METHODS

This was a case control study design conducted from April to September, 2018 on 180 participants ranging from 18-65 years, age and sex matched after obtaining ethical approval from research and ethics committee, Federal Teaching Hospital Gombe (ref: NHREC/25/10/2018). Sociodemographic data, type and duration of treatment with cART were obtained using a standardized self-administered questionnaire. One hundred and twenty were HIV sero-positive subjects on cART and 60 were HIV-sero-positive treatment naïve as control group. Three aliquots of 5mls of blood samples were collected from ante-cubital vein under aseptic technique separately into three ethylenediamine tetra-acetic acid (EDTA) sample bottles for HIV viral load (HIV-mRNA), complete blood count (CBC) and CD4 cell count respectively, after obtaining informed consent from all the participants. CD4 cell count was obtained from the BD-FACS Analyzer, while the HIV viral load was assessed using real time PCR machine (COBAS Ampliprep and COBAS Tagman-96 by Roche). The haematological parameters (CBC) were obtained using haematology auto analyzer Sysmex KX-2 1N (five-part differential). In addition to that, peripheral blood film was made and stained using Leishman's stain for morphological studies. The procedure for all the analyses was followed as indicated in the manufacturer's user manual.

The data obtained from the participants was entered into a spread sheet and analysed using IBM SPSS version 20.0.0 (IBM Corp. 2011). Categorical variables such as age group, sex distribution and clinical history were presented as proportions using bar charts. Quantitative variables such as age, duration of HIV and HAART therapy, CD4 count, haematological variable, such as packed cells volume (PCV), haemoglobin (Hb) concentration, total white blood cells count (WBC), platelet count, differential and viral load, were also presented as mean and standard deviation. Comparison of categorical variables between various study groups was done using chi-square statistics. For continuous variables, mean difference between two groups was compared using Student's t test, while comparison among more than two groups was conducted using ANOVA. Pearson's correlation was used to assess association between CD4 count cell, haematological variables, viral load and duration of cART therapy. Probability value of less than 0.05 was considered statistically significant in this study.

RESULTS

A total number of 180 participants were enrolled into this current study comprising of 120 PLHIV subjects on cART and 60 age and sex-matched seropositive treatment naïve controls. There were 99 (82.5%) of subjects placed on first and third regimens, while 21 (17.5%) were on second regimen. The mean age of the participants was 36.1years (±11.6) with a range of 18-65 years. Male constituted 48.9% while females 51.

People living with HIV infection (PLHIV) on cART had significantly increased ($p < 0.05$) Hb (13.1 ± 1.9g/dl), PCV (0.36 ± 0.05l/l) MCV (94.0 ± 11.1f/l) MCH (33.7 ± 5.0pg), MCHC (35.6 ± 1.9g/dl) and platelet count (264.0 ± 106.0 x 10^9/l) when compared with treatment naïve Hb (11.2 ± 2.9g/dl), PCV(0.32 ± 0.07l/l), MCV (81.4 ± 7.5fl), MCH (28.7 ± 7.5pg), MCHC (33.9 ± 3.6g/dl) and platelet count (245.6 ± 108.8 x 10^9/l). There was no statistically significant difference observed in total white blood cell count of the two groups (Table 1).

There was also no statistically significant difference in the haematological variables observed based on the types of cART among treatment experienced group, except for basophils ($p = 0.026$; Figure 1).

People living with HIV infection on cART for more than 12 months had the highest CD4 count (651 ± 249 cells/µl), while 1-6months had the lowest CD4 count (536 ± 228 cells/µl). Viral load count was seen to be highest among those on cART for 1-6months (1064 ± 31.0 cp/ml), while lowest value was observed in 12 months and above (42.74 ± 6.9 cp/ml) (Table 2).

People living with HIV on cART for 6-12months had the highest platelet count, while 3-6months had the lowest count as seen in the Figure 2.

DISCUSSION

The findings from this present study showed that, basically the response to combination antiretroviral therapy (cART) was generally good among people living with HIV infection (PLHIV) on cART. This positive effect observed among those taking cART could be due to mean reduction in viraemia resulted from the positive effect of the antiretroviral therapy, which allows the generation of naïve CD4 and haemopoietic progenitor cells through cell division. Patients on cART had significantly higher CD4 count when compared with treatment naïve controls. This is commensurate to other similar findings, where the percentage of CD4 cell count was reported to have increased from 12.9% to 23.7% after six months on cART.[10,11]

Table 1: Comparison of the mean CD4 count and viral load of 120 PLHIV on cART based on duration of treatment

Duration on cART	**CD4 count (cells/uL)**	**Viral load (cp/ml)**	**P-value**
More than 12 months	651 (± 249)	42.74 (± 74)	0.020
6 to 12 months	607 (± 347)	112 (± 15.8)	0.021
1 to 6 months	536 (± 228)	1064 (±31.0)	0.026

Statistical significance set at P ≤ 0.05 (using student test followed by Turkey post hoc test)

Haematological measured variables such as haematocrit (PCV), haemoglobin concentration (Hb), monocytes count, MCV, MCH and MCHC were observed to be significantly increased among PLHIV on cART when compared with treatment naïve PLHIV. The increase in some of these haematological variables in this study may likely be due to the reduction in viral load, decreased destructions of mature haematopoietic cells of multiple lineages, relieve of bone marrow from suppression by the mediated abnormal cytokines generated from the direct infection by the HIV infection itself and improvement in the blunted erythropoietin response among PLHIV on cART.[12] These findings are in contrast with studies by other researchers, who have reported abnormal decrease in haematocrit, haemoglobin concentration[13,14], red cell indices and total RBC count, leading to anaemia among PLHIV on cART.[15] Possible explanation to these differences of the results might likely be due to differences in the cART regimen, side effect, environmental factors, differences in study population, socio-demographic characteristics of the study subjects and methodology. The significant increase in MCV and macrocytosis observed among people on cART, may be essentially due to inhibition of cellular DNA synthesis by the effect of cART, which results in impaired synthesis of erythrocytes precursor cells and delayed nuclear maturation in the bone marrow subsequently leading to macrocytosis.[16,17] The haematocrit, haemoglobin concentration, RBC counts were significantly lower among treatment-naïve in this current study. This is probably due to decreased RBC production by the direct effect of HIV infection itself on the bone marrow and other haemopoietic organs, effect of opportunistic infection, decreased production of erythropoietin, increased destruction of RBC as a result of mediated autoimmune antibodies on the red cells and nutritional deficiencies such as vitamins and iron deficiencies.[18] This finding is in agreement with previous studies reported by Adane *et al* (2012) which showed 49.5% of the sample population had anaemia [19]. This finding is also in accordance with the study reported by Enawgaw (2014) and Akinbami (2010), which also reported anaemia among treatment-naïve patients. [7,18] Basophil count was significantly higher among people on cART when compared with treatment naïve patients. This increase in the relative percentage of basophils among PLHIV on cART might probably be due to allergic reaction caused by the side effect of the drugs. [20] This finding is in accordance with studies by Enawgaw (2014) and Jiang (2015), which reported an increase in basophil counts.[7,21] Other haematological variables also did not differ statistically in

Table 2: Comparison of haematological variables of PLHIV on cART and treatment naïve controls

	HIV Status		
Parameter	**PLHIV on cART n = 120**	**Treatment Naïve PLHIV Controls n = 60**	**P-value**
PCV (l/l)	0.36 ± 0.05	0.32 ± 0.07	<0.001
Haemoglobin (g/dl)	13.1 ± 1.9	11.2 ± 2.9	<0.001
Total WBC (x10 ^{9}/l)	5.31 ± 1.61	5.49 ± 2.77	0.569
RBC (x10 ^{12}/l)	3.98 ± 0.67	3.79 ± 0.98	0.002
Platelet (x10 ^{9}/l)	263.9 ± 106.0	243.3 ± 108.1	0.223
Neutrophil (%)	47.7 ± 12.3	50.2 ± 19.2	0.229
Neutrophil (abs)	2.61 ± 2.38	3.01 ± 1.39	0.090
Lymphocyte (%)	47.25 ± 11.97	43.8 ± 17.4	0.117
Lymphocyte (abs)	2.44 ± 0.75	2.12 ± 0.96	0.081
Monocyte (%)	2.53 ± 1.45	1.67 ± 0.86	<0.001
Monocytes (abs)	0.132 ± 0.084	0.132 ± 0.148	0.066
Basophil (%)	0.05 ± 0.22	2.47 ± 2.05	0.015
Basophil (abs)	1.87 ± 0.04	0.89 ± 0.20	0.073
Eosinophil (%)	2.53 ± 1.9	0.25 ± 0.8	0.001
Eosinophil (abs)	0.131 ± 0.098	0.090 ± 0.073	<0.001
MCV (fl)	94.0 ± 11.1	81.3 ± 7.1	<0.001
MCHC (g/dl)	35.6 ± 1.9	33.94 ± 3.63	<0.001
MCH (pg)	33.7 ± 5.0	28.8 ± 7.6	<0.001

Statistical significance set at P ≤ 0.05 (using student t test followed by Turkey post hoc)

this current study, however, PLHIV on cART for over six to 12 months had significantly higher platelet counts than those who have been on cART for 3-6 months.

An improvement in platelets count, while on cART seen in this study, may be due to a decrease in non-specific deposition of circulating immune complexes on platelets or a decrease in viraemia leading to little or no destruction of megakaryocyte by direct HIV infection. This is consistent with other studies that reported significant increase in platelet count over time among PLHIV on cART.[22,23] A study by Leticia *et al* (2014) in Southeastern Nigeria, reported an increase in the median platelet count after six months by about 9.4% among PLHIV on cART [23].

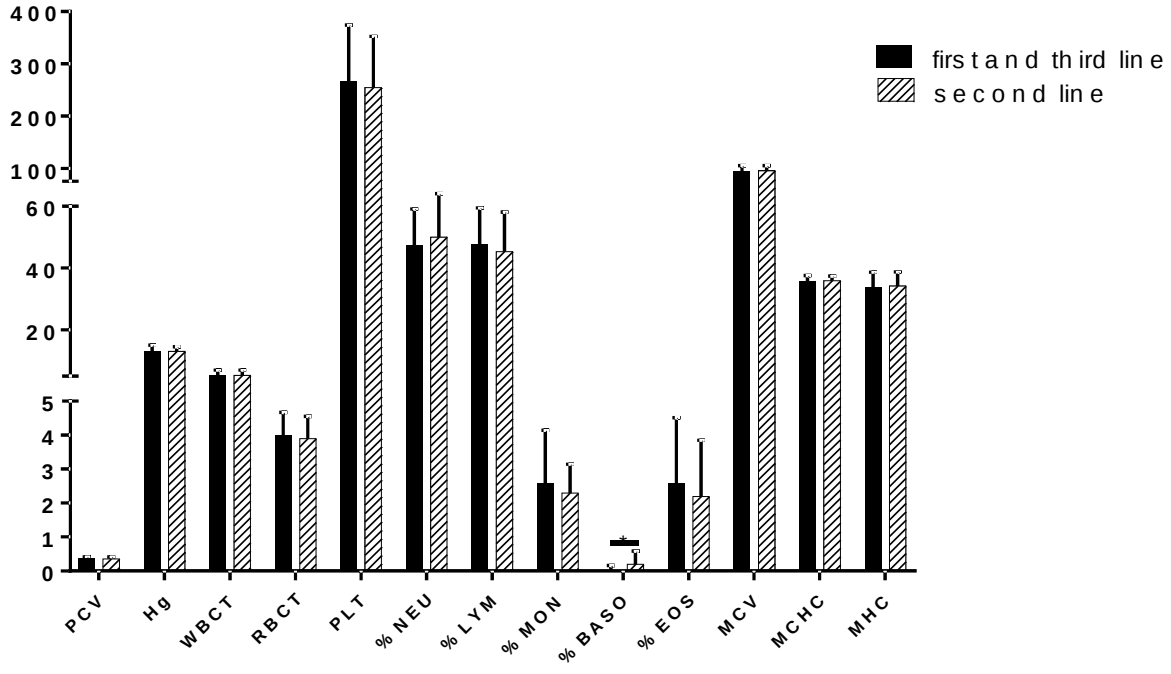
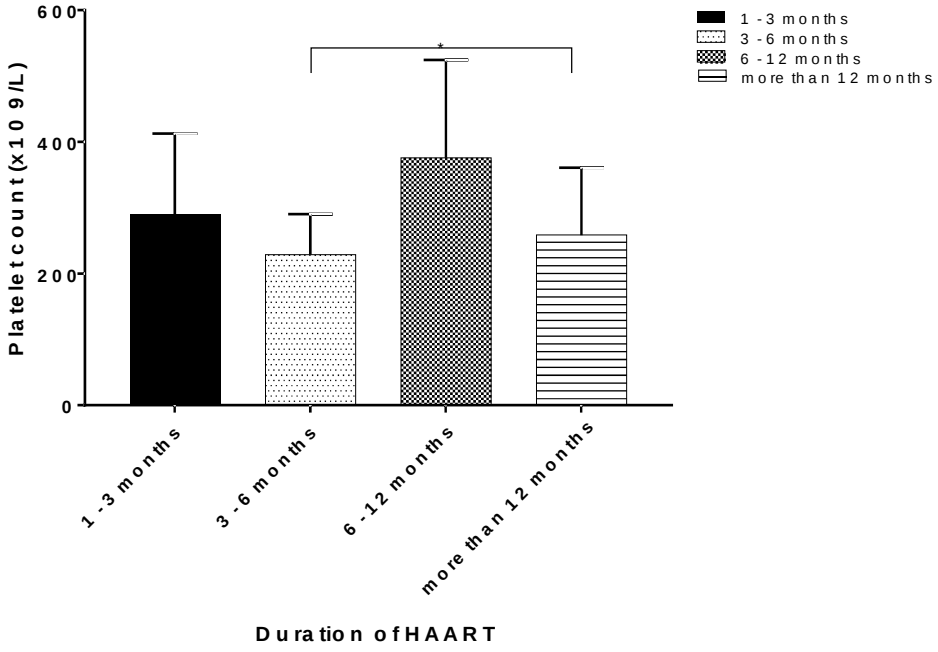


Figure 1: The distribution of haematological parameters of PLHIV based on first and third line (n = 99) and second line (n = 21) cART regimens (* p = 0.026)



(*statistically significant; p = 0.002)

Figure 2: Platelet count variation of 120 PLHIV based on duration of cART

The overall improvement in the haematological abnormalities seen among PLHIV on cART, in this study and other findings may be attributed to the positive effect of cART on the reduction of viral load, decreased destructions of mature haemopoietic cells of multiple lineages, blunted increased in erythropoietin, resulting in the restoration of the immune status among PLHIV on cART.

CONCLUSSION

This study confirms that combination antiretroviral therapy has a positive effect on some haematological variables, reduces viral load and restores some haematopoietic activities. The changes in the haematological variables were similar for patients on the different cART regimens.

Conflict of Interest: None

Authors' Contributions:
All authors have participated in all stages of the research, from conceptualization, data collection, data analysis to the development of the manuscript.

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