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Persistent Anaemia and Unresolved Systemic Dysfunction among Persons Living with HIV Infection in Southern NigeriaEuphoria C. Akwiwu^{1*}, Dennis A. Abunimye¹, Josephine O. Akpotuzor¹

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Author for Correspondence *: ecakwiwu@gmail.com/ +234-803-677-7296/ORCID Number: 0000-0001-6097-557X, <https://dx.doi.org/10.4314/sokjmls.v9i2.35>**Abstract**

Anaemia in human immunodeficiency virus (HIV) infection has remained a significant morbidity indicator affecting both disease progression and outcome. Its persistence despite antiretroviral therapy continues to generate interest. Reports of systemic dysfunction in association with HIV has necessitated exploring possible systemic indices for assessment and monitoring of the infection. In resource-poor settings such as the study locality, identifying affordable biomarkers for disease management is important. This study was a case-control, analytical single-site research among people living with HIV in Southern Nigeria. Purposive sampling technique was used to enrol equal numbers of newly diagnosed HIV-infected persons, patients on antiretroviral therapy and control subjects. Ethical considerations were duly obtained. Red cell parameters and CD4 count were assessed by automation. Aminotransferase enzyme activities were assayed by standard colorimetric methods. De Ritis ratio was calculated. The RBC, Hb and HCT were significantly reduced in HIV subjects on HAART compared to those newly diagnosed and control subjects. Both MCV, RDW-SD and De Ritis ratio were high in newly diagnosed subjects compared to HIV subjects on HAART and control subjects, while the CD4 count varied significantly across all three groups. There were significant moderate negative correlations between De Ritis ratio and both mean cell volume and mean cell haemoglobin. Overall systemic state rather than the index of immune response correlated with indices of anaemia among persons living with HIV infection and

receiving antiretroviral therapy.

Keywords HIV, antiretroviral therapy, anaemia, immune response**Introduction**

Impairment in the red blood cell production capacity and accelerated removal from circulation are at the forefront of the pathogenicity of anaemia. In specific terms however, the mechanisms are a lot more complex and interrelated such that defective production could among other things reduce functional capacity as well as signal early destruction (Akwiwu *et al.*, 2024a; Volberding *et al.*, 2004). Investigations into these diverse but related aspects of anaemia have trailed the search for better management of HIV infection and its progression to AIDS (Kyeyune *et al.*, 2014; Kallianpur *et al.*, 2016). Anaemia in HIV infection has remained a significant morbidity indicator affecting both disease progression and outcome. Its persistence despite antiretroviral therapy has not gone unnoticed but has continued to shape the protocol for medical care and management. Constant review of antiretroviral agents in this light is among the follow-up interventions readily observed (Denué *et al.*, 2013; Kallianpur *et al.*, 2016; Panwar *et al.*, 2016). Previously, marrow suppression and haemolytic predisposition have been linked to the anaemia of HIV infection. Progressive research into HIV-related anaemia in the study area observed anaemia from marrow suppression was higher in proportion compared to that from haemolysis when comparable number of participants exposed to combinations

with agents capable of marrow suppression and haemolysis were compared (Okafor *et al.*, 2019). The study attributed such a pattern to the fact that haemolysis had the potential of inducing erythropoiesis unlike marrow suppression.

Additionally, ineffective utilization of nutritional components for red cell synthesis such as iron has been reported among the HIV population in the study locality (Okafor *et al.*, 2016). Such findings reflecting dysregulation at the systemic level has occasioned exploring systemic indices available for assessment and monitoring of disease conditions. In resource-poor settings such as the study locality where individual socio-economic status hugely influences access to medical care, late hospital presentation and uncontrolled health conditions due to financial constraint remain challenges in disease management (Akwiwu *et al.*, 2020; Ndem *et al.*, 2021). Thus, known derivatives of routinely assessed parameters are beginning to prove useful, particularly if such variables are found to be useful in disease assessment (Akpan *et al.*, 2023; Akwiwu *et al.*, 2024b). De Ritis ratio, a derivative of the aminotransferases, and its implications are being studied for different inflammatory conditions. It is appreciated as an index of pathophysiology and its possible utility in disease conditions is worth exploring.

Materials and Methods

Ethical approval was sought and duly obtained from the University of Calabar Teaching Hospital Health Research Ethics Committee, while informed consent was obtained from each participant. The present study was carried out among a total of 90 subjects at the University of Calabar Teaching Hospital at Calabar, Southern Nigeria. At a national prevalence of 1.4% among persons of reproductive age, minimum sample size of approximately 21 was obtained for the study. Purposive sampling technique was used to enrol 30 newly diagnosed HIV-infected persons, 30 persons living with HIV infection on antiretroviral therapy and 30 apparently healthy HIV sero-negative persons as normal control. Uniform gender ratio was ensured across the groups during enrolment. The subjects were between 20 and 45 years of age. Granting of consent, not being on medication for at least 3

months prior to the study except for the group on antiretroviral therapy and being relatively stable in health were considered as inclusion criteria. Volunteers to the study who could not meet the inclusion criteria were excluded.

Blood sample was appropriately obtained from each subject for testing of red cell parameters, CD4 count, aspartate transaminase (AST) and alanine transaminase (ALT). De Ritis ratio was mathematically derived as the ratio of AST to ALT. Red cell parameters and CD4 count were determined by automation using Mindray BC-5000 Haematology Analyzer (Mindray Medical International Limited, China) and Partec CyFlow Counter (Sysmex Corporation, Japan). Enzyme activities of AST and ALT were determined by colorimetric method using kits from ELITechGroup clinical systems, France.

Data generated were entered into Microsoft excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) software version 22.0. Frequencies, one-way analysis of variance and Pearson's correlation were used for analysis of data. The least significant difference (LSD) Post Hoc Test accompanied the ANOVA for interpretation of significant variance. Statistical significance was drawn at a $p < 0.05$.

Results

In terms of HIV status of the participants, equal numbers per group were purposively enrolled. Those on HAART were made up of 33.3%, those that were newly diagnosed were 33.3%, while sero-negative control subjects were also 33.3%. Females constituted 60% of the subjects, while males were 40% across all three groups. Assessed red cell parameters included; Red blood cell count (RBC), Haemoglobin concentration (Hb), Haematocrit (HCT), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Mean cell volume (MCV), Red cell distribution width as standard deviation (RDW-SD), Red cell distribution width as coefficient of variation (RDW-CV).

The RBC, Hb and HCT were significantly reduced in HIV subjects on HAART compared to those newly diagnosed and control subjects. Both MCV,

RDW-SD and De Ritis ratio were high in newly diagnosed subjects compared to HIV subjects on HAART and control subjects, while the CD4 count varied significantly across all three groups as seen in Table 1. More than half (60%) of the subjects on therapy were anaemic (Figure 1).

Pearson's correlation of measured parameters among the subjects on therapy revealed significant moderate negative correlations between De Ritis ratio and both mean cell volume and mean cell haemoglobin ($p = 0.004$ and $p = 0.008$ respectively) (Figures 2 and 3).

Table 1. Parameters of HIV subjects on HAART, newly diagnosed and control subjects

Parameters	HIV subjects on HAART (n = 30)	HIV subjects newly diagnosed (n = 30)	Control subjects (n = 30)	P-value
RBC ($\times 10^{12}/l$)	4.24 \pm 0.62*	4.53 \pm 0.77	4.77 \pm 0.63	0.012
Hb (g/l)	120.27 \pm 18.28*	131.27 \pm 18.11	134.60 \pm 13.75	0.004
HCT (l/l)	0.35 \pm 0.04*	0.38 \pm 0.05	0.39 \pm 0.04	0.006
MCV (fl)	79.93 \pm 10.75	85.37 \pm 9.98*	79.99 \pm 8.32	0.051
MCH (pg)	27.71 \pm 4.41	29.25 \pm 3.69	27.92 \pm 3.62	0.264
MCHC (g/l)	345.30 \pm 16.45	341.47 \pm 14.08	347.80 \pm 14.85	0.285
RDW-SD (fl)	44.65 \pm 4.88	45.95 \pm 7.76*	42.25 \pm 3.11	0.039
RDW-CV (%)	14.22 \pm 2.25	13.91 \pm 2.08	13.86 \pm 2.49	0.802
CD4 (cells/ml)	425.27 \pm 237.38*	413.13 \pm 200.28*	621.67 \pm 142.16	0.001
ALT (U/L)	26.93 \pm 12.19	22.80 \pm 10.81	26.97 \pm 8.04	0.215
AST (U/L)	32.83 \pm 15.06	32.13 \pm 16.39	30.43 \pm 8.01	0.783
De Ritis	1.27 \pm 0.38	1.43 \pm 0.38*	1.19 \pm 0.38	0.058

Key: **RBC** = Red blood cell, **Hb** = Haemoglobin, **HCT** = Haematocrit, **MCV** = Mean cell volume, **MCH** = Mean cell haemoglobin, **MCHC** = Mean cell haemoglobin concentration, **RDW-SD** = Red cell distribution width (as standard deviation), **RDW-CV** = Red cell distribution width (as coefficient of variation).

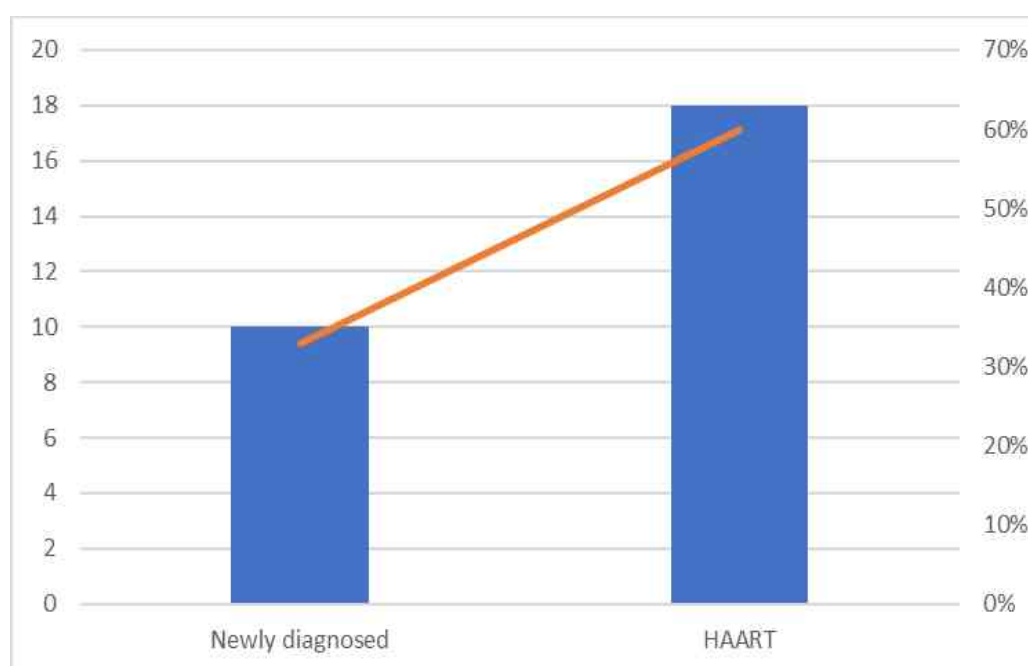


Figure 1. Prevalence of anaemia among study participants living with HIV

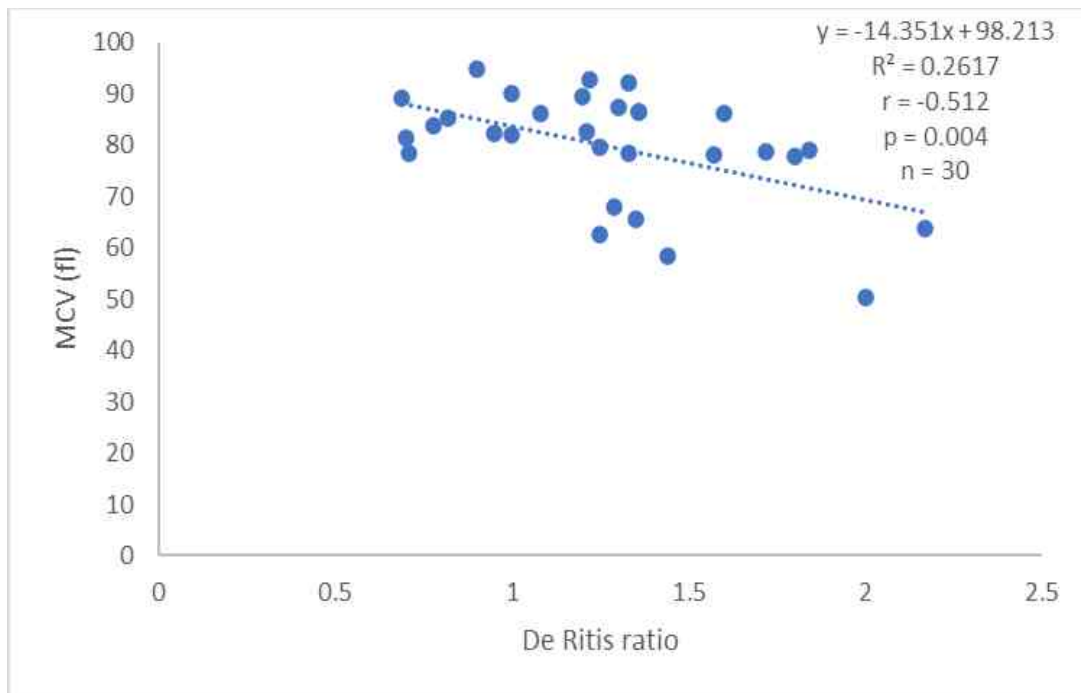


Figure 2. Correlation between De Ritis ratio and mean cell volume among subjects on antiretroviral therapy

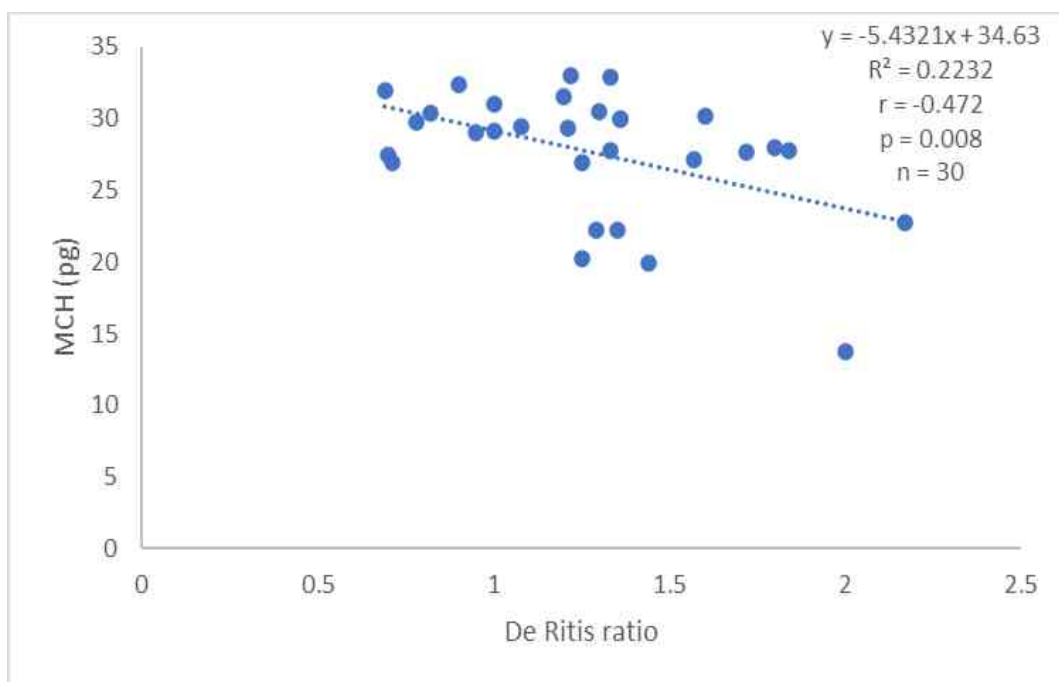


Figure 3. Correlation between De Ritis ratio and mean cell haemoglobin among subjects on antiretroviral therapy

Discussion

Management of HIV infection with antiretroviral agents has since progressed from monotherapy to the combined model approach of highly active antiretroviral therapy (HAART). Administration of treatment has also been adjusted to commence as soon as possible following diagnosis, while periodic monitoring is encouraged (Akpotuzor *et al.*, 2013; Abunimye *et al.*, 2022). Improvements in the form of increased life expectancy and the lowering of transmission risks are part of the gains of these strategies (Wandeler *et al.*, 2016; WHO, 2022). However, there are reports of anaemia and associated pathologies despite HAART (Kallianpur *et al.*, 2016; Panwar *et al.*, 2016; Al-Kindi *et al.*, 2017). The present study also observed anaemia with a higher prevalence among subjects on therapy. In assessing for associating parameters to the observed anaemia, the CD4 count, and De Ritis ratio were considered. The CD4 count has been traditionally used as an indicator of immune state and response to the invasive debilitating mechanism of the virus. In the present study, the CD₄ count varied significantly across the groups of the control subjects, HIV subjects on HAART treatment, and newly diagnosed subjects. The mean count for those on treatment was higher than that for the newly diagnosed, while the control group had the highest mean count. Although the marker of immune state improved with antiretroviral therapy, anaemia which is an important factor for overall survival was not seen to be ameliorated after the pattern of the CD4 count.

This study observed no statistical relationships between the CD4 count and anaemia-related parameters but recorded negative correlations between the later and De Ritis ratio. De Ritis ratio has been linked to wide state anaerobic glycolysis and has played a role in understanding of cancer pathophysiology, thus serving as an index of systemic dysfunction (Kawamoto *et al.*, 2012; Ruhl and Everhart, 2012; Liu *et al.*, 2014; Ozaki *et al.*, 2020; Udosen *et al.*, 2022). Increasing De Ritis ratio related with both reducing mean cell volume and mean cell haemoglobin. Microcytic hypochromic anaemia arises from different mechanisms including poor iron incorporation during erythropoiesis. This could be from nutritional iron deficiency or iron sequestration as part of the pathophysiology in the anaemia of chronic diseases. The later has been previously observed and reported from the study locality (Okafor *et al.*, 2016). Addressing systemic deregulation, both from the infection and drug interaction may be at the heart of

ameliorating anaemia in HIV infection. Among persons on therapy therefore, an associating systemic marker could be helpful. This is quite desirable if the marker is routinely assessed or derivable from routine tests.

In conclusion, improved immune response does not necessarily relate with the amelioration of anaemia in HIV infection. Thus, evaluating overall systemic state in addition to assessing the index of immune response should be considered in the management of HIV-associated anaemia.

Conflict of Interest

Authors declare no conflict of interest

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