Niger. J. Physiol. Sci. 41 (June 2026): 9 – 15
www.njps.physiologicalsociety.com
www.ojshostng.com/index.php/njphysiologicalsciences

Niger. J. Physiol. Sci.

Full-Length Research Article

Changes in Serum Erythropoietin and Haematological Profile of HAART-Experienced HIV Patients Living with Type 2 Diabetes Mellitus in South-South Nigeria

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Summary: Human immunodeficiency virus (HIV) infection, HIV medications and type 2 diabetes mellitus adversely affect kidney function, including erythropoiesis. However, studies evaluating the combined effects of these conditions on serum erythropoietin (EPO) levels and the haematological profile of affected individuals in southern Nigeria are scarce. In this study, 128 adults aged 18 to 59 years were selected from designated health facilities in southern Nigeria. They were divided into four groups (n=32 persons per group). Fasting/random blood sugar (FBS/RBS) levels, complete blood count (CBC), glycated haemoglobin (HBA1C), and serum levels of erythropoietin were determined using standard methods. Results show a significant increase ($P < 0.05$) in serum EPO and total red blood cell count (TRBC) in the HIV-only group compared with the other groups. The total white blood cell count (TWBC), platelet count, and platelet-to-lymphocyte ratio increased in the diabetic-plus-HIV group. The FBS/RBS and the HBA1C levels increased in the diabetic and diabetic plus HIV groups compared to others. Increased erythropoiesis, depicted by increased serum EPO and TRBC, could be attributable to improved use of HIV medications and improved lifestyle among the HIV positive patients on treatment. Poorly controlled T2DM with resultant increased HBA1C levels in the diabetic and diabetic plus HIV groups was responsible for the increase in TWBC and platelet count, likely due to impaired immune function leading to sepsis in these groups.

Keywords: *erythropoietin, erythropoiesis, type 2 diabetes mellitus, HIV, highly active anti-retroviral therapy.*

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Manuscript received: April 2026; Accepted: June 2026

DOI: https://doi.org/10.54548/njps.v41i1.2

INTRODUCTION

The longevity of HIV-positive patients has increased significantly since the advent of HIV medications, with an increased 7-year survival probability among patients on HAART as high as 83.9 % (Girum *et al*., 2024). However, increases in age and immunosuppression increase their susceptibility to age-related metabolic disorders, including type 2 diabetes mellitus (T2DM) and infections (Lin *et al*., 2018). This scenario is known to cause several physiological changes affecting erythropoiesis and glucose homeostasis, leading to anaemia and a persistent hyperglycemic state. Several changes also occur in blood indices and erythropoietin (EPO) levels, resulting in higher susceptibility to infections (Abu-Ashour *et al*., 2017). HIV infection also results in opportunistic infections in these patients (Teeka *et al*., 2024). HIV and T2DM individually and in synergy can affect haematological parameters through chronic inflammatory changes and bone marrow suppression, leading to immune and metabolic dysfunctions, which is related to the poor HbA1c levels indicating poor glycaemic control (Singh *et al*., 2020).

Chronic hyperglycemia from impaired glucose tolerance leads to increased metabolic disruptions, which is worsened in HIV positive patients on HAART (Avari and Davendra, 2017)6, and this can affect haematological and cellular functions, leading to an abnormal haematological profile. Differences abound in the ways the patients' clinical characteristics aggravate or reduce the haematological manifestations, resulting in dichotomous effects on the haematological profile, which could worsen or improve anaemia (Huibers *et al*., 2020).

EPO produced by the kidneys is responsible for red blood cell (RBC) production (erythropoiesis). However, T2DM reduces kidney function, which may include alterations in erythropoietin levels. Several studies in Nigeria have shown the prevalence of HIV patients on ART, with baseline T2DM prevalence ranging from 2.3 % (Isa, 2016)8 to 7.5 % (Enya, 2023)9 which results in premature death of one third of HIV patients in that category (Martin, 2024). Furthermore, studies investigating serum EPO levels

and hematological indices in HIV- positive patients with T2DM are scarce. Therefore, this study was designed to investigate the changes in serum EPO and hematological indices in HIV treated patients HAART- (Highly active antiretroviral therapy) experienced living with T2DM in southern Nigeria.

METHODS

Study Design: This was a case-control study. Sample size was determined using the formula shown in Equation 1 (Kaur, 2017).

$$N = \frac{z^2 \text{pq}}{d^2} \qquad \text{(Equation 1}$$

Where N = the desired sample size, Z = the standard normal deviate, usually set at 1.96, which corresponds to the 95 % confidence interval. p = the proportion in the study population estimated to have both HIV infection and diabetes. Based on previous studies, diabetes prevalence among HIV-infected adults was reported to be 10.3 % (Alfonso and Hernandez, 2017) in place of as reported in the National Representative Survey (2009-2010). The sample size in this study was extrapolated from this value at 95 % confidence and a 5% error margin.

q = 1.0 – P, d = Degree of accuracy desired, usually set at 0.05

$$\text{N} = \frac{1.96^2 \text{ x } 0.09 \text{ x } 0.91}{(0.05)^2}$$

N = 125.8 |approximately 126.

Dividing this by 4 groups is 31.5, so a total of 128 subjects were recruited for this study so that each group had 32 patients (since 31.5 participants is impractical).

Study Area: The study was conducted in designated hospitals in Akwa Ibom State. Akwa Ibom is a state in the south-south zone of Nigeria with a land area of 7,249 square kilometres. It lies between Latitudes 4°32' and 5°33' North and longitudes 7°35 and 8°25' East. It is bounded by Rivers State, Cross River State, Abia State, and the Gulf of Guinea on the East, West, North, and South, respectively. It has an estimated population of 7.2 million, with an annual growth rate projected at 3.2%.

Study Population: The study population were HIV-seropositive adult patients on HAART who were also diabetics seen within the study period attending the HIV/AIDS clinics in Immanuel General Hospital and Polyclinic in Eket, General hospitals Iquita in Oron and Ikot Ekpene, Cottage hospitals in Ibeno and Ikot Ekpene Udo, Saint Luke's Hospital in Anua as well as normal patients from Samaritan Clinic/Hospital in Ek*et al*l in Akwa Ibom State.

Study Period: The study was carried out over a period of six months, from December 2023 to May 2024.

Collection and Treatment of Samples: The socio-demographic profile of the patients were obtained with the aid of an interviewer-administered questionnaire and this included age, sex, occupation, marital status and educational status. A focused medical history, family history, and physical examination was also done before sample collection.

Anthropometric Measurements: The height of the participants was measured with a stadiometer stabilized against a vertical wall or with tape without shoes or caps with the patients standing erect on a hard surfaced of the stadiometer, head, buttocks and feet touching the vertical wall, with the head level with the horizontal plane (Warrier *et al*., 2023).[13] The participants were asked to place their legs together, bringing the ankles or knees together, whichever came together first (often they came together simultaneously). The height was read to the nearest 0.5 cm. The weight was measured with a digital weighing scale. The participants stood over the center of the scale with their body weight evenly distributed between their feet without shoes. The arms were made to hang freely by the sides of the body, with palms facing the thighs, the head held up and face forward. The weight was read to the nearest 0.5 kg (Warrier *et al*., 2023).[14] The body mass index was calculated from weight/ height^2 (Kg/M^2).

The blood pressure (BP) was measured on the left arm with a mercury sphygmomanometer at heart level, using an appropriate cuff size. The subjects were allowed to relax for 5 minutes in a seated position before their blood pressure was measured. Blood pressure readings were recorded to the nearest whole number, and the average of the three recordings computed.

Laboratory Investigations: The patients were educated on the procedure for vene-puncture and some of the complications which include some pain at the insertion site, tingling sensation if a nerve is penetrated mistakenly and a wheal at the surrounding area due to oedema. They were re-assured of caution and carefulness in the procedure. For each patient, after selecting the site in the upper limb with prominent veins, a 10 mL syringe with needle was used to collect the blood. Eight milliliters of blood was collected from every case and control for laboratory analysis.

Analyses of Samples:

Blood samples of the patients were analyzed using different methods.

Full Blood Count Determination: This was carried out using the SYSMEX K21 N haematology auto-analyzer which functions on the principles of light scatter (for cell counting) and colorimetry for hemoglobin concentration. As the cells flow in a linear pattern across a light source, there is forward and sideways scatter of light. The scatter gram generated will depict the size and granularity of the cells which will enable characterization of the cells.

Glycated Hemoglobin Determination: The procedure utilizes a weak binding cation-exchange resin for the rapid separation of glycated hemoglobin (HBA1C) from all the other hemoglobins. A hemolyzed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbA0 binds to the resin. HbA0 consist of all the other hemoglobins except HBA1C which remains in solution. After the mixing period, a filter is used to separate the supernatant containing the HBA1C from the resin. The percent glycol-hemoglobin is determined by measuring the absorbance at 415 nm of the HBA1C fraction and the total hemoglobin fraction. The

ratio of the two absorbance gives the percent of HBA1c (Chaila *et al*., 2021).[15]

Erythropoietin Assay: ELISA Test Principle: Erythropoietin standards or samples were added to the micro ELISA plate wells and combined with specific antibodies. Then a biotinylated detection antibody specific for Human erythropoietin and avidin-horshradish. Peroxidase (HRP) conjugates was added to each microplate well and incubated. Free components were washed away.
The substrate solution was then added to each
well. Only those wells containing human erythropoietin biotinylated detection antibody and avidine-HRP conjugates turned blue. Stop solution was then added to the enzyme substrate to terminate the reaction with resultant yellow colour. The optical density (OD) measured spectrophotometrically using microplate reader at 450 nm wavelength which represents the concentration of human erythropoietin (Obeagu *et al*., 2019).

The concentrations of human erythropoietin in the samples were then calculated by comparing the OD of the samples to the standard curve

Statistical Analyses
The data obtained were analysed using both descriptive and inferential statistics. The values obtained were reported as Mean±standard deviation, and significant differences in means were examined using One-way Analysis of Variance (ANOVA). Additionally, Duncan's test was used to separate the means, and data were analysed using the Statistical Package for the Social Sciences (SPSS version 20.0) and GraphPad Prism 7.0.

Ethical considerations
Ethical approval for this study was obtained through a written request to the Health Research Ethics Committee of The University of Uyo as well as the Human Research and Ethics Committee of Akwa Ibom State Ministry of Health with protocol number (HREC No. **AKHREC/22/2/24/213**). Armed with the ethical approvals, the Heads of the various hospitals involved were consulted and their permission obtained. Written informed consent was obtained from each patient, signed or thumb-printed. Patients were free to withdraw from participating in the study at any point without any negative implication. Patients were not identified by their names but initials were used. Patient's data as well as records from this study were kept with strict confidentiality both in hard copy and spread sheets (soft copy). Patients found to have abnormal results were informed and referred to appropriate specialists.

RESULTS

Total Red Blood Cell (TRBC) of Participants in the Various Groups: The result in Figure 1 showed significant increase ($p < 0.05$) in TRBC count in the HIV only group compared with other groups.

Haemoglobin Concentration (HB) of Participants in Various Groups: Figure 2 shows no significant difference in Haemoglobin concentration among the groups ($p > 0.05$).

Mean Corpuscular Volume (MCV) of Participants in Various Groups: Figure 3 shows that there was no significant difference in the mean corpuscular volume (MCV) of participants in various groups ($p > 0.05$).

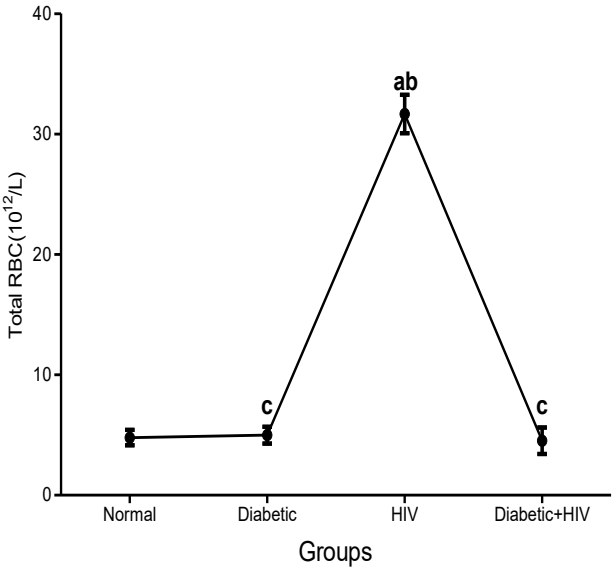

Figure 1
Total Red Blood Cells (RBC) of participants in the Various Groups

Mean Corpuscular Hemoglobin (MCH) of Participants in Various Groups: Figure 4 shows that there was no significant difference in the mean corpuscular hemoglobin (MCH) of participants in various groups ($p > 0.05$).

Mean Corpuscular Haemoglobin Concentration (MCHC) of Participants in Various Groups: Figure 5 shows that there was no significant difference in the mean corpuscular haemoglobin concentration (MCHC) of participants in various groups ($p > 0.05$).

Erythropoietin Levels of Participants in Various Groups: Figure 6 shows that serum EPO was significantly higher ($p < 0.05$) in the HIV-only group compared with other groups.

Total White Blood Cell Count (WBC) of Participant in all the Groups: The result in Figure 7 shows a significantly higher level of TWBC was found in the diabetic plus HIV group compared with other groups ($p < 0.05$).

Neutrophil/Lymphocyte Ratio of Participants in Various Groups: Figure 10 shows no significant difference in the Neutrophil/Lymphocyte Ratio among participants across groups ($p > 0.05$).

Platelet Count of the Participants in Various Groups: The results in Figure 11 show that platelet levels were significantly increased ($p < 0.05$) in the diabetic plus HIV group compared with other groups.

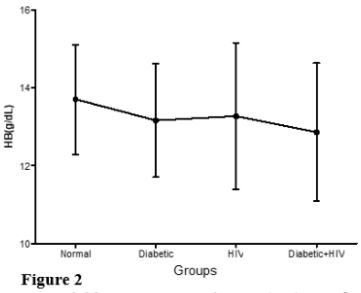

Figure 2
Hemoglobin Concentration (HB) of Participants in Various Groups

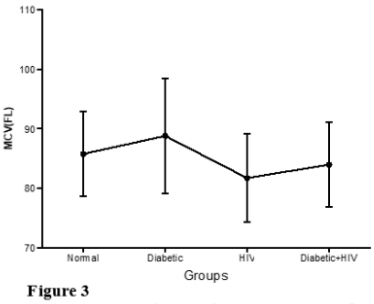

Figure 3
Mean Corpuscular Volume (MCV) of Participants in Various Groups

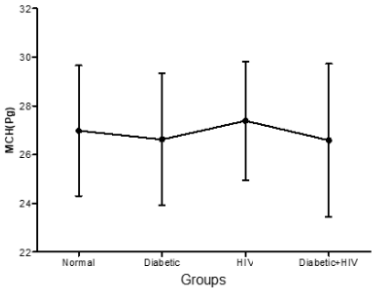

Figure 4
Mean Corpuscular Haemoglobin (MCH) of Participants in Various Groups

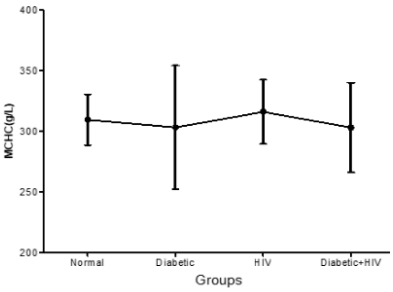

Figure 5
Mean Corpuscular Haemoglobin Concentration (MCHC) of Participants in Various Groups

Lymphocyte Count of Participants in all Groups: Figure 9 shows no significant difference in lymphocyte counts across groups ($p > 0.05$).

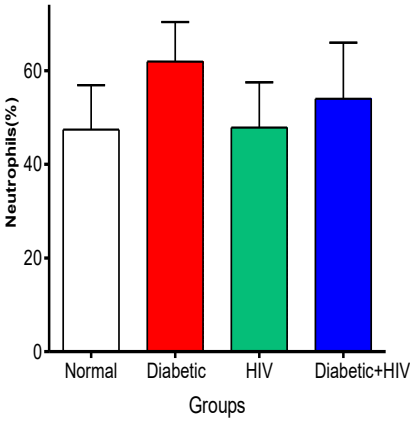

Figure 8.
Neutrophil Count of Participants in all the Groups

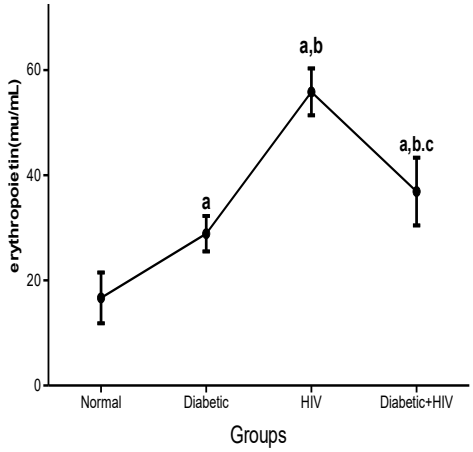

Figure 6
Erythropoietin Levels of Participants in Various groups

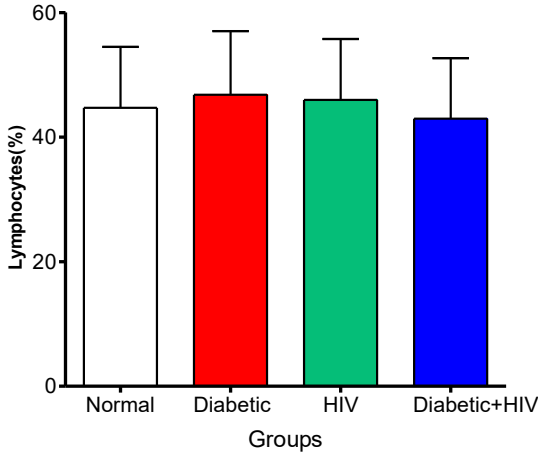

Figure 9
Lymphocytes Count of Participants in all the Groups

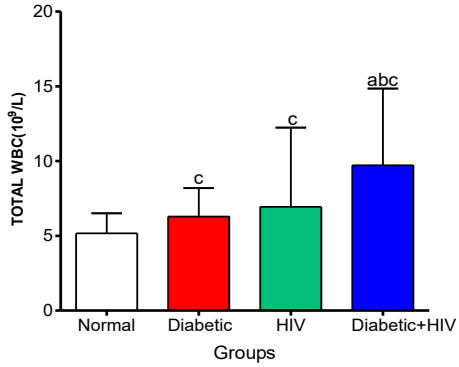

Figure 7
Total White Blood Cell Count (WBC) of Participants in all the Groups

Neutrophil Count of Participants in all Groups: Figure 8 shows no significant difference in neutrophil count among the groups ($p > 0.05$).

Platelet/Lymphocyte Ratio of Participants in Various Groups: Figure 12 shows that the platelet/lymphocyte ratio was also significantly increased ($p < 0.05$) in the diabetic plus HIV group compared with other groups.

Fasting/Random Blood Sugar Levels of Participants in Various Groups: The result in Figure 13 shows that there was a significant increase ($p < 0.05$) in FBS/RBS in the diabetic and the diabetic plus HIV groups compared with other groups.

Glycated Haemoglobin (HBA1C) Level of Participants in Various Groups: The mean HBA1C level also increased significantly ($p < 0.05$) in the diabetic and the diabetic plus HIV groups, as seen in Figure 14.

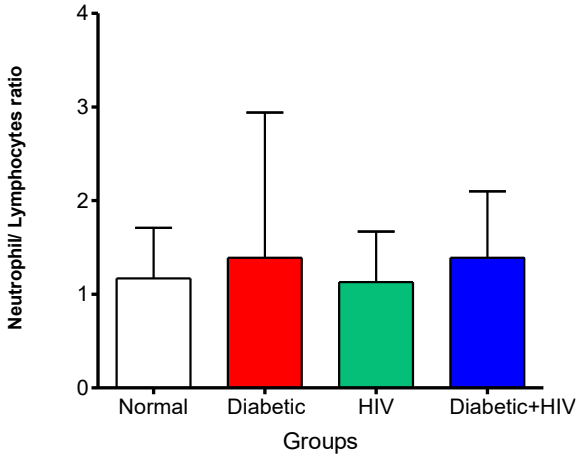

Figure 10.
Neutrophil- Lymphocytes Ratio of Participants in Various Groups

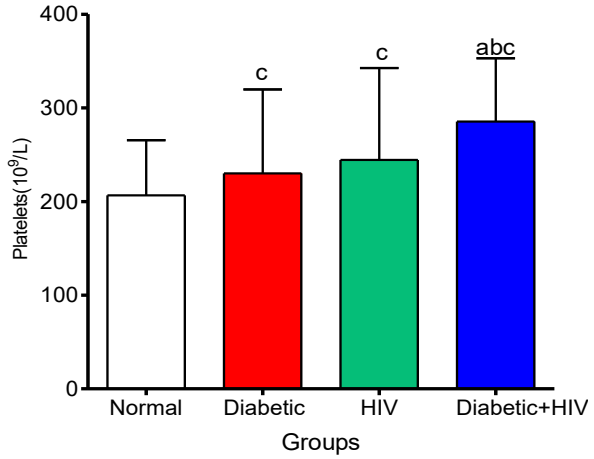

Figure 11
Platelet Count of the Participants in Various Groups

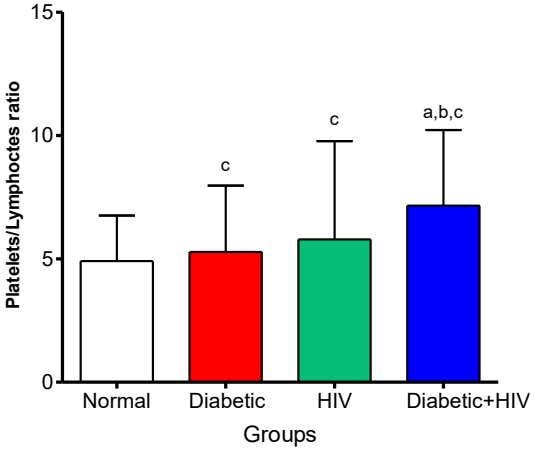

Figure 12
Platelet/Lymphocyte Ratio of Participants in Various Groups

DISCUSSION

The significant increase in TRBC count in the HIV-only group could be explained by the concomitant increase in the EPO level found in this study. Hypoxia caused the increase in serum EPO, due to low partial pressure of oxygen affecting the kidneys (Schoener and Borger, 2024). However, in certain conditions of hypoxia with extremely low partial pressure of oxygen, like in severe covid 19, serum EPO level decreases, and this could be traced to the uncoupling of physiological hypoxia signalling circuit resulting in the 'hypoxia paradox'. (Begemann *et al*., 2021).

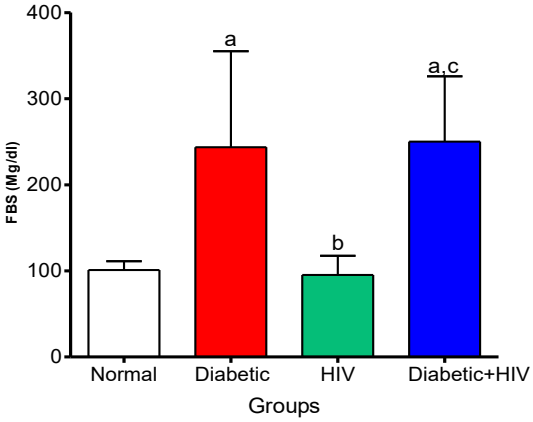

Figure 13
Fasting/Random Blood Sugar Level of Participants in Various Groups

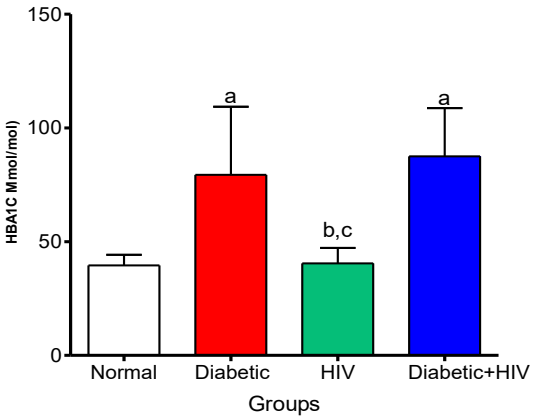

Figure 14
Glycated Hemoglobin (HBA1C) Level of Participants in Various Groups

Increased EPO due to hypoxia could be regarded as a compensatory mechanism in the HIV group who are normally prone to having aneamia from bone marrow suppression from specific anti-retroviral drugs, poor nutrition as well as chronic inflammatory processes and immune mediated cell destruction. However, with the use of improved HAART therapy, which could result in reduced viral load and increased CD4 count as well as the general stability of the health of the patients, a rise in reticulocyte count could possibly contribute to the rise in total RBC count with a resultant decrease in hemoglobin as a rise in erythrocyte count may indicate anemia depending on the bone marrow status (Omosigbo *et al*., 2024)19 or a normal hemoglobin concentration as seen in participants in the HIV group. Increased EPO levels can cause stress induced erythropoiesis with resultant reticolocytosis (Fattizzo *et al*., 2019).

The findings from this study is in contrast with another study where it was discovered that HIV-1 appeared to suppress the synthesis of EPO in patients, however, EPO

could be a good intervention in managing anemia among people living with HIV (Obeagu and Obeagu, 2024). A positive association between CD4 counts and EPO level following commencement of HIV medications was also found in another study (Okolo *et al*., 2022). This could explain the increase in total RBC count obtained in the HIV group in this study.

The EPO level in the diabetic and the diabetic plus HIV groups in this study are also significantly higher than that obtained in the normal group. This indicates that diabetes also contributes to increase in erythropoietin level but this does not translate to increase in the total RBC and other red cell indices such as HB/PCV, MCV, MCH and MCHC levels. The increased EPO level in the diabetic groups could be due to diabetic induced chronic kidney damage which is associated with poor survival in diabetic patients (Fujita *et al*., 2019). This which could lead to decrease EPO clearance and increase production which can subsequently reduce blood glucose level through improved blood glucose metabolism. This study shows significant increase in TWBC, platelets and platelet/lypmphocytes ratio in the diabetic plus HIV group compared to all other groups.

These findings agree with a study in which platelet and WBC count were significantly higher in diabetic patients than in the controls (Adane *et al*., 2021). This was traceable to advanced glycation end products (AGEs), oxidative stress, angiotensin II, and cytokines activation which play fundamental role in inflammation. Increased platelet count as seen in the diabetic plus HIV group in this study is further supported by another study in which it was traced to the increased in surface adhesion molecules P-selectin as decrease in fasting glucose level was associated with decreased expression of P-selectin activation markers (Mastrogiacomo *et al*., 2023). Increase in platelets count and total WBC as seen in the diabetic plus HIV group in this study could also be explained due to the increased erythropoietin level in both HIV and diabetic plus HIV groups as erythropoietin has been implicated in increasing the viscosity of blood (Brar *et al*., 2021). with resultant stasis from increased red cell mass which could eventually predispose the patients to infection and inflammation. This also applies to the platelet/lymphocytes ratio since there was no significant different in this ratio in the various groups. These findings suggest that improving blood glucose control may improve WBC and platelet function.

In this study the diabetic and diabetic plus HIV groups were found to have significantly higher fasting/random blood sugar levels which also corresponded with the increased glycated hemoglobin. This agrees with the findings of increased glycated hemoglobin level in the diabetic and diabetic/HIV comorbid patients as seen in this study. Increased glycated hemoglobin due to poorly controlled diabetes mellitus could also be linked to the presence of hypocalcaemia and hyponatreamia (Preyanka *et al*., 2020). Poor glycaemic control increased HBA1C level in the diabetic and diabetic plus HIV groups as found in this study is also noted to be associated with critically ill patients (Luethi *et al*., 2016). This was associated with increased urea and creatinine levels in the diabetic group compared to other groups (Ikpe *et al*., 2025) as well as a worse renal profile which further explains the reduction in serum EPO level in the diabetic only group in this study when compared to the HIV groups. Therefore, the higher the glycated hemoglobin level, the worst the prognosis.

In conclusion, the present study suggests that HIV increases erythropoiesis by increasing serum EPO levels as well as TRBC count in HAART-experienced patients; this could lead to reticulocytosis and eventually polycythemia. Also, T2DM increases the risk of infections via the increase in white blood cell indices and platelet count, especially in patients with diabetes and HIV comorbidities.

This study is limited to HIV and type 2 diabetic patients in hospitals in Akwa Ibom State in southern Nigeria. Similar studies should be carried out by non-governmental organizations to cover the entire country or other specific regions.

Acknowledgements
The authors are grateful to the academic and technical staff of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Uyo, for their technical support.

Authors Contributions
The study was conceived and designed by Itoro Lawrence Ikpe and Christopher Edet Ekpenyong who also wrote the original draft. Uduak A. Inwang made important corrections and rearrangement of the work. Samson Timothy Kayode analyzed and interpreted the data.

Declaration of Conflicts of Interests
The authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

Data access statement
Data obtained from this study cannot be shared due to ethical reasons, as most of the participants declined third-party sharing of their data due to the sensitivity of their illness.

Funding
The authors of this article self-funded this research work.

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