Research Article

Evaluation of Haematocrit, Haemoglobin Level and EPO mRNA Expression among Male Blood Donors in Ibadan, South-West Nigeria

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ABSTRACT

Evaluating erythropoietin (EPO) mRNA expression alongside conventional haematologic indices offers deeper insight into the body's compensatory erythropoietic response. While most studies on blood donors focus on haemoglobin (Hb) and haematocrit (Hct), little is known about EPO gene expression, particularly among male donors who constitute the majority in many regions. This cross-sectional study assessed Hct, Hb, and EPO mRNA expression in 100 male donors in Ibadan, Nigeria (50 regular, 50 occasional). Haematological parameters were measured using automated methods, while EPO mRNA was quantified by GAPDH-normalized RT-PCR. Data were analysed using independent t-tests, Pearson's correlation, and Mann–Whitney U tests at $p<0.05$ level of significance. Regular donors had significant lower mean Hct (31.86±6.32%) and Hb (10.59±2.11 g/dL) compared to occasional donors (41.40 ± 3.30% and 13.77±1.10 g/dL; $p<0.001$). The EPO mRNA expression was more variable among regular donors, with 68% showing downregulation and 32% upregulation, whereas occasional donors had 10% downregulation and 90% upregulation ($p = 0.905$). The EPO mRNA and Hct showed a weak, insignificant correlation ($r = 0.102$, $p = 0.315$). Regular donors were more likely to exhibit subclinical anaemia, suggesting that Hb screening alone may not detect early haematologic stress and erythropoietic compensation. Occasional donors had higher EPO gene expression compared to regular donors, indicating they may be well suited for blood donation due to their enhanced ability to regenerate red blood cells. These findings underscore the possibility of including molecular markers like EPO mRNA in donor monitoring to enhance donor safety and eligibility assessment.

Keywords: Blood Donation; Erythropoietin; Gene Expression; Haematocrit; Haemoglobin

INTRODUCTION

Blood donation remains one of the most essential pillars of contemporary medical practice, forming the backbone for transfusion medicine and emergency healthcare systems worldwide. Every year, millions of lives are saved through timely blood transfusions during surgeries, childbirth, accidents, and treatment of haematological disorders such as anaemia and leukaemia. Despite these benefits, the act of blood donation can induce transient physiological changes in donors, particularly in red cell indices and iron metabolism, which if recurrent, may result in subclinical anaemia or impaired erythropoiesis (Ogar *et al.*, 2022). In countries such as Nigeria, where the demand for blood greatly exceeds the supply, a considerable proportion of donors are regular (or commercial), raising concerns about the long-term haematologic impact of repeated phlebotomy.

Haematocrit (Hct) and haemoglobin (Hb) are two of the most widely used indicators for assessing the haematologic status of individuals and are critical for evaluating the safety of both donors and recipients. Haematocrit measures the volume of packed red blood cells (RBCs) relative to whole blood cells (Mondal *et al*., 2024), while haemoglobin measures the oxygen-carrying capacity of the blood. Frequent blood donation, especially without adequate recovery time, has been linked to gradual reductions in these parameters, predisposing donors to anaemia and iron deficiency. Studies conducted in Nigeria have repeatedly reported significantly lower Hb and Hct levels among regular donors compared to first-time or voluntary donors (Nwogoh *et al.,* 2012; Okunade *et al*., 2024). This consistent pattern suggests that economic motivation and repeated donations without proper monitoring may negatively affect donor's health.

While Hb and Hct serve as frontline indicators for assessing donor eligibility, they only provide a snapshot of the current haematologic state and do not reveal the molecular mechanisms that regulate erythropoiesis after blood loss. The hormone erythropoietin (EPO) plays a pivotal role in stimulating red blood cell production in response to hypoxia or anaemia. The EPO is primarily produced in the kidneys, where peritubular interstitial cells detect reduced oxygen tension and initiate its synthesis (Haase, 2013). The expression of EPO at the messenger RNA (mRNA) level is particularly significant, as it directly reflects the transcriptional activation of the EPO gene mediated by hypoxia-inducible factors (HIFs). The quantification of EPO mRNA provides a molecular window into how the body responds to blood loss, complementing traditional haematologic indices in assessing erythropoietic capacity.

Messenger RNA (mRNA) mediates protein synthesis by copying genetic information from DNA and carrying it to ribosomes (Saldana *et al.,* 2024). It determines the rate and efficiency of protein synthesis, influencing physiological responses such as erythropoiesis and cellular adaptation to hypoxia. In the context of blood donation, assessing EPO mRNA expression levels provides valuable insight into the transcriptional and translational activity driving red blood cell regeneration. Following blood loss, hypoxia-inducible pathways stabilize HIF-1α, which binds to the promoter region of the EPO gene to enhance transcription (Haase, 2013). The resulting increase in EPO mRNA synthesis serves as a compensatory response to stimulate erythroid progenitor cells within the bone marrow.

However, individual variability in EPO gene expression has been observed, influenced by genetic factors, nutritional status, and donation frequency (Vocanec *et al.,* 2019). While a number of genome-wide association studies (GWAS) could link RBC traits such as RBC size, hemoglobin (Hb) content and hematocrit levels to single-nucleotide polymorphisms (SNP), only few studies analyzed circulating EPO levels (Corre *et al.,* 2021). This variability implies that some donors may exhibit efficient molecular compensation following blood loss, while others experience inadequate erythropoietic recovery, leading to chronic anaemia or fatigue, further emphasized that repeated blood donation without sufficient time for physiological recovery can blunt EPO responsiveness, thereby reducing the rate of red blood cell regeneration. Therefore, integrating molecular markers such as EPO mRNA into donor evaluation could enhance early detection of erythropoietic strain before haematologic indices fall below eligibility thresholds.

In Nigeria, screening of blood donor typically focuses on haemoglobin levels and serological testing for transfusion-transmissible infections (TTIs) such as HIV, hepatitis B, and hepatitis C (Akanmu *et al.,* 2019; Okafor, Onifade *et al.,* 2022). While these measures are indispensable for ensuring safety, they provide limited insight into the donor's physiological capacity to recover post-donation. The minimum haemoglobin threshold recommended for donation in Nigeria is 13.5 g/dL for males and 12.0 g/dL for females (WHO, 2023). However, studies have shown that donors with haemoglobin values near the lower limits may still experience significant reductions in iron stores and slower haematologic recovery. Regular blood donations can engender iron depletion and its complications; reducing the prevalence of iron depletion among blood donors is a key strategy for optimizing donors' health (Oyedeji *et al.,* 2020). These observations highlight the need for additional markers that can better reflect erythropoietic function, such as molecular quantification of EPO mRNA expression.

Ibadan, the capital of Oyo State, represents one of the most active blood donation centers in southwestern Nigeria. The region's blood donor population is influenced by diverse socioeconomic factors, including poverty and lack of education, which contribute to the predominance of commercial donors. Health disparities in donor populations across Nigeria necessitate location-specific studies to understand physiological responses within particular contexts. Hence, examining haematologic indices and EPO mRNA expression among male donors in Ibadan is not only scientifically valuable, but also regionally relevant, providing data that can inform national donor safety policies.

The biological significance of studying EPO mRNA expression extends beyond blood donation. Use of EPO is not only for haematopoietic regulation, but also as a multifunctional hormone with cytoprotective, angiogenic, and anti-apoptotic effects (Cornelio *et al.,* 2022). The gene's expression is tightly controlled by oxygen availability through HIF-dependent transcriptional mechanisms. Under hypoxia, reduced oxygen tension stabilizes HIF-α, which binds with HIF-β to form an active transcriptional complex that enhances EPO mRNA synthesis (Haase, 2013). Understanding the dynamics of this molecular mechanism among blood donors provides insights into how the body adapts to transient anaemia caused by donation.

MATERIALS AND METHODS

Study Design

This research adopted a cross-sectional experimental design aimed at evaluating Haematocrit, haemoglobin levels, and erythropoietin (EPO) mRNA expression among male blood donors in Ibadan, South-West Nigeria. The cross-sectional approach was chosen because it allows the simultaneous assessment of both haematologic and molecular parameters within a defined population at a specific point in time. This design is particularly suitable for comparing groups that differ in donation frequency, regular versus occasional donors while controlling for confounding demographic and physiological variables. Cross-sectional studies in haematologic assessment are advantageous for establishing baseline differences between donor categories without requiring longitudinal follow-up. The design was also ethically appropriate, considering that all participants were apparently healthy male donors and no intervention was introduced beyond routine phlebotomy and blood sampling.

Study Area

The study was conducted in Ibadan, the capital city of Oyo State, located in the South-West geopolitical zone of Nigeria. Ibadan serves as one of the major centers for blood donation and transfusion services in the region. The city hosts multiple government and private hospitals, diagnostic centers, and blood banks that attract donors from diverse socioeconomic and occupational backgrounds.

The choice of Ibadan as the study area was based on its high donor activity and accessibility to blood donors from multiple donation centers, including tertiary hospitals and organized mobile blood drives. Moreover, the population's diversity in lifestyle, nutrition, and donation patterns makes it ideal for evaluating variations in haematologic and molecular responses. The study environment also provided the necessary laboratory facilities for both haematological and molecular analyses.

Study Population

The study population consisted exclusively of apparently healthy male blood donors aged between 18 and 65 years who presented voluntarily or commercially for blood donation at selected donation centers in Ibadan. Female donors were excluded to eliminate confounding effects of menstrual blood loss and hormonal variation on haematologic parameters.

Sample Size and Sampling Technique

A total of 100 male blood donors were recruited using a purposive sampling technique, which allowed deliberate inclusion of individuals fitting the defined donor categories. This sample size was considered adequate for molecular and haematologic analysis and also the statistical comparison between two independent groups, as supported by previous haematologic studies (Nwogoh *et al.,* 2012; Abdulsalam *et al.*, 2024). Purposive sampling was selected because the population of interest, active male donors was specific and accessible only within blood bank and donation centers. Recruitment was conducted under the supervision of transfusion service personnel to ensure compliance with donor eligibility criteria.

A total of 100 participants were enrolled, divided equally into two categories based on donation frequency:

Regular donors (n = 50): individuals who had donated blood at least three times within the preceding twelve months.

Occasional donors (n = 50): individuals who had donated fewer than three times within the same period.

Ethical Approval and Informed Consent

Ethical clearance for the study was obtained from Oyo State Ministry of Health Ethical Committee. Written informed consent was obtained from all participants before data collection. Participation was voluntary, and confidentiality of information was maintained throughout the study. The study protocol adhered to the principles of the Declaration of Helsinki (2013) on human subject research. Participation was voluntary, all participants were fully informed of the study's purpose, procedures, potential risks, and benefits. Confidentiality was maintained throughout, and participants were assigned unique identification codes instead of personal names. Written informed consent was obtained prior to sample collection.

Sample Collection, Preparation and Handling

For specimen collection and preparation, suitable tubes or collection containers were used. Five (5) ml of venous blood samples was collected into an Ethylene diamine tetracetic acid (EDTA) bottle. The sample was used for haematologic analysis while the remaining portion was centrifuged at 4000rpm for 5mins to obtain plasma which was dispensed into RNA stabilization reagent tubes and stored at −20°C to preserve RNA integrity prior to molecular analysis of EPO mRNA.

Haematological Analysis

Haematologic parameters, haemoglobin (Hb) concentration and haematocrit (Hct) were determined using automated hematology analyzers following standard operational protocols. The results were interpreted according to the World Health Organization (2024) reference standards, with anaemia defined as Hb < 13.0 g/dL and Hct < 39% for adult males.

Determination of Haematological Parameters (Haematocrit and Haemoglobin)

The three-part Mindray haematology analyzer was used to perform the haematological parameters. Additionally, Wallace H. developed the Coulter principle, which serves as the foundation for the test's concept. According to Coulter's theory from the late 1940s, when particles pulled through an orifice while an electric current is flowing through it, the impedance changes in proportion to the volume of the particle passing through the orifice. The particle's displacement of the electrolyte is the source of the impedance pulse. However, a three-part hematology analyzer performs its job using both flow cytometry and Coulter's concept. The measuring of the quantity and properties of cells is known as cytometry. Cell size, cell count, cell morphology (including form and structure), cell cycle phase, DNA content, and the presence or lack of particular proteins on the cell surface or in the cytoplasm during flow are among factors that are assessed in cytometry. A method for identifying and quantifying the physical and chemical properties of a population of cells or particles is called cytometry. Clinical anaemia was defined using the World Health Organization cut-off point of < 13 g/dL for men, while subclinical anaemia was defined as haemoglobin values of 13 to 13.9 g/dL for men (ogar *et al.*, 2022)

Molecular Analysis of Erythropoietin (EPO) mRNA Expression

The level of EPO mRNA expression was measured using real-time polymerase chain reaction (RT PCR). The principle of Polymerase Chain Reaction (PCR) is the in vitro exponential amplification of a specific target DNA sequence through repeated cycles of temperature-dependent reactions following RNA extraction. It involves separating DNA strands with heat (denaturation), binding short DNA primers to the template DNA at lower temperatures (annealing), and synthesizing new DNA strands by a DNA polymerase enzyme at an optimal temperature (extension). This process is repeated many times, exponentially increasing the copies of the target DNA segment for analysis. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001). Results were categorized as upregulation or downregulation based on fold-change differences relative to control (occasional donors).

PCR Result Interpretation

NTC (negative control without template): If the CT value of NTC is greater than 35 or there is no CT, there is no contamination in the system; if the CT value is less than 35, the system may be contaminated or the primers are not specific, forming primer-dimers, and cleaning experiments are recommended environment, change the sterile water, check the

primers for contamination, or appropriately reduce the primer concentration, and optimize the primer design.

Amplification curve and CT value: The standard amplification curve is a smooth "S" type. Generally, the Ct value of the target gene is between 20 and 30, and the Ct value of the internal reference is between 15 and20. If the Ct value is small, it is recommended to dilute the template; if the Ct value is large, it is recommended to increase the template concentration or increase the reaction system; if the Ct value is greater than 35, the test result cannot be used for quantitative analysis of gene expression, but it can be used for qualitative analysis.

Melting curve: The standard melting curve presents a single peak. If there are double peaks or multiple peaks in the melting curve, there may be contamination, primer dimer or non-specific amplification. It is recommended to reduce the primer concentration or optimize the primer design.

Data Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 27 Descriptive statistics (mean, standard deviation, and percentage distribution) were computed for all variables. Independent sample t-tests were used to compare mean Hb and Hct values between regular and occasional donors. Mann–Whitney U test was used to analyze non-parametric variables such as EPO mRNA fold-change expression. Pearson's correlation analysis assessed the relationship between EPO mRNA, haematocrit, and haemoglobin levels. The level of significance was set at $p < 0.05$.

RESULTS

Regular donors exhibited significantly reduced haematocrit (Hct) and haemoglobin (Hb) values compared to occasional donors ($p < 0.001$), suggesting that repeated blood donation is associated with a measurable decline in red cell indices. Results in Table 1 demonstrate a statistically significant difference between donor categories, confirming that regular donors had mean hematologic indices below the WHO threshold for adult males (Hb < 13 g/dL; Hct < 39%).

Erythropoietin (EPO) mRNA Expression Patterns

Quantitative analysis of erythropoietin (EPO) mRNA expression revealed variable transcriptional responses between the donor categories. Using the ΔΔCt quantification method, relative expression was categorized as upregulated or downregulated relative to the reference group (occasional donors). Although EPO mRNA downregulation predominated among regular donors, the group difference was not statistically significant ($p = 0.905$). This outcome suggests a complex pattern of erythropoietic response to recurrent donation. The high proportion of downregulation among regular donors could reflect either desensitization of renal EPO synthesis pathways or exhaustion of erythroid response capacity. In contrast, the strong upregulation among occasional donors indicates efficient short-term compensatory adaptation following single or infrequent donations.

To evaluate the relationship between molecular and haematologic parameters, Pearson's correlation analysis was performed between EPO mRNA expression and both haematocrit and haemoglobin levels.

Table 1: Comparison of Haematologic Indices Between Regular and Occasional Donors

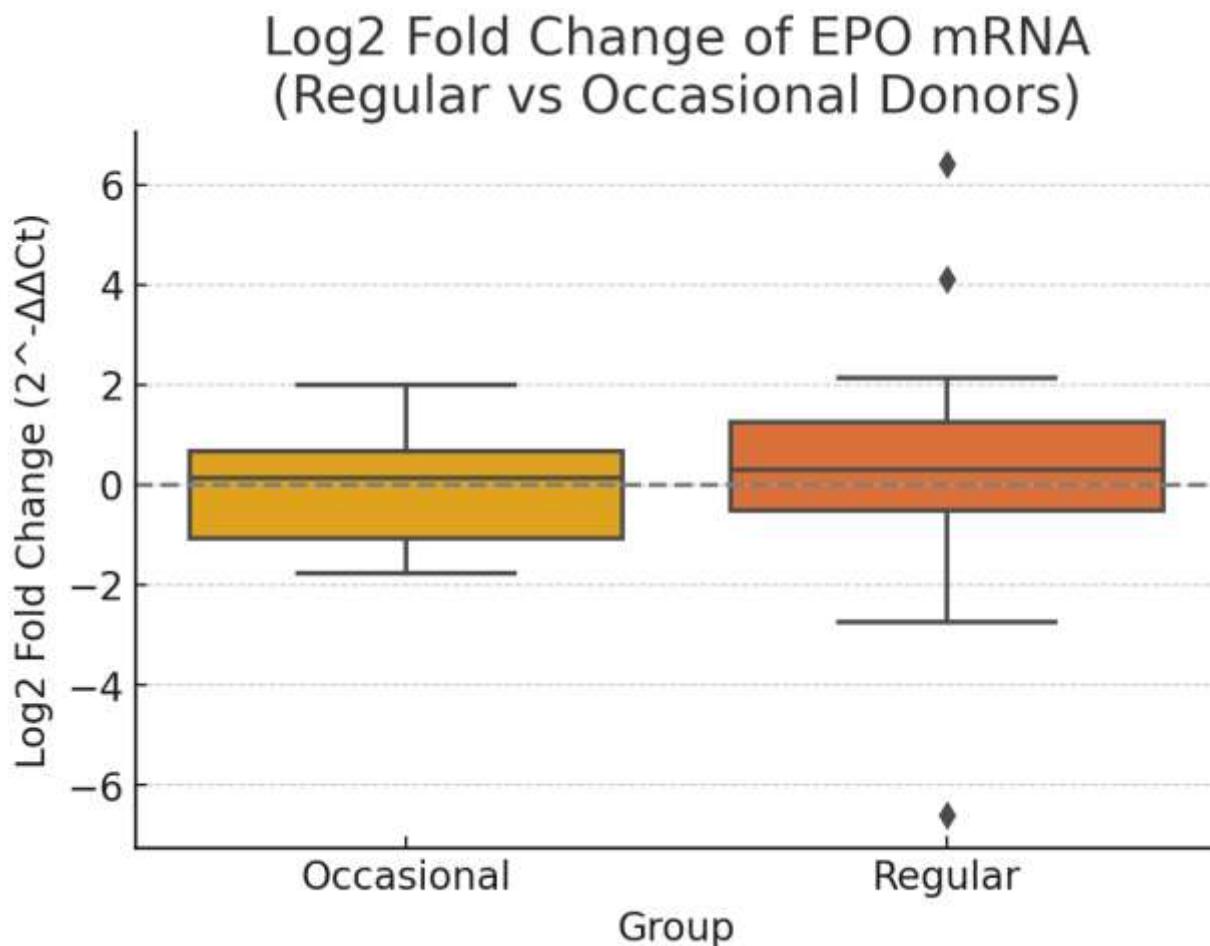| Parameter	Regular Donors (Mean ± SD)	Occasional Donors (Mean ± SD)	t-value	p-value
Haematocrit (%)	31.86 ± 6.32	41.40 ± 3.30	8.97	<0.001*
Haemoglobin (g/dL)	10.59 ± 2.11	13.77 ± 1.10	8.65	<0.001*

Independent t-test significant at *p* < 0.05.

Figure 1: EPO mRNA Expression in Regular and Occasional Blood Donors

Table 2: Correlation Between EPO mRNA Expression, Haematocrit, and Haemoglobin Levels

Variable	r-value	p-value
EPO mRNA vs. Haematocrit	0.102	0.315
EPO mRNA vs. Haemoglobin	0.086	0.354

The analysis revealed weak positive but non-significant correlations between EPO mRNA and both haematocrit and haemoglobin

DISCUSSION

The present study investigated haematocrit, haemoglobin, and erythropoietin (EPO) mRNA expression among male blood donors in Ibadan, South-West Nigeria, comparing regular and occasional donors. The findings revealed that regular donors exhibited significantly reduced haematocrit and haemoglobin levels compared to occasional donors, and that most regular donors showed downregulation of EPO mRNA expression. These outcomes suggest that repeated blood donation imposes haematologic stress and may attenuate molecular responses involved in erythropoiesis.

The significant difference in haematocrit and haemoglobin between donor categories indicates that repeated blood donation exerts measurable effects on red cell indices. Regular donors had mean haematocrit and haemoglobin values of 31.86 ± 6.32% and 10.59 ± 2.11 g/dL, respectively, compared to 41.40 ± 3.30% and 13.77 ± 1.10 g/dL among occasional donors (Table 1). These findings are consistent with previous studies in Nigeria and other sub-Saharan African populations, which have

consistently reported reduced haematologic parameters among regular donors (Nwogoh *et al.,* 2012; Ogar *et al.,* 2022).

Frequent donation leads to recurrent depletion of iron stores and red blood cell mass (Oyedeji *et al.,* 2020). Unless sufficient recovery time and dietary supplementation occur, cumulative deficits can persist and manifest as reduced haemoglobin and haematocrit. Regular donors, especially those who donate for financial reasons, are less likely to receive post-donation nutritional support, thereby increasing susceptibility to subclinical anaemia.

The findings also agree with those of (Jeremiah, 2010), who reported that iron loss associated with repeated donation often surpasses the physiological rate of replacement, leading to reduced erythrocyte count and microcytic hypochromic anaemia in chronic donors. Such evidence underscores that donor frequency directly influences haematologic integrity and suggests the importance of implementing longer intervals between donations.

The prevalence analysis showed that 58% of regular donors had haematocrit below the normal limit and 54% had haemoglobin below 13 g/dL, confirming that more than half of regular donors exhibited subclinical anaemia and were not fit for blood donation. Subclinical anaemia can compromise donor well-being, leading to fatigue, reduced physical performance, and decreased oxygen-carrying capacity (Tsai *et al.,* 2019).

These findings reinforce earlier reports that frequent donors require more comprehensive post-donation evaluation. According to WHO (2023), the minimum recommended haemoglobin level for male donors is 13.0 g/dL; however, repeated donors may begin donation with borderline values and progressively decline with each subsequent session. The current study highlights the importance of introducing extended recovery periods or supplementation programs to restore erythropoietic balance among repeat donors.

Erythropoietin is a principal regulator of erythropoiesis, synthesized primarily in the kidneys in response to hypoxia. Following blood donation, transient reduction in oxygen-carrying capacity normally stimulates renal production of EPO, thereby promoting erythroid proliferation in the bone marrow (Haase, 2013). The expected physiological response is an increase in EPO gene transcription and protein secretion. However, the downregulation observed among regular donors suggests that repeated donation might suppress or desensitize this feedback mechanism.

The transcriptional complex hypoxia inducible factor (HIF) plays a central role in the maintenance of oxygen (O_2) homeostasis, which is essential for cell survival (Ginouvès *et al.,* 2008). Chronic low-level hypoxia in frequent donors could lead to adaptive attenuation of the hypoxia-inducible factor (HIF) pathway, thereby reducing transcriptional activation of the EPO gene. In other words, the renal cells become less sensitive to oxygen fluctuations due to prolonged exposure, leading to diminished EPO response. This may partly explain why some regular donors fail to recover haematologic parameters promptly after donation.

In contrast, the predominance of EPO upregulation among occasional donors indicates an intact compensatory mechanism that enables efficient erythropoietic recovery after single or infrequent donations. This dichotomy in gene expression highlights the value of EPO mRNA quantification as a molecular biomarker of erythropoietic capacity.

The dissociation between EPO mRNA expression and haematologic restoration may be explained by the multi-layered control of erythropoiesis. While EPO transcription represents the initial molecular signal, the eventual increase in haemoglobin and haematocrit depends on iron availability, bone marrow responsiveness, and erythrocyte lifespan (Haase, 2013). Thus, a donor with adequate EPO transcription but low iron stores may still show poor haematologic recovery.

Another plausible explanation is temporal lag: EPO mRNA upregulation occurs rapidly after hypoxic stimulus, but measurable haematologic improvement follows days or weeks later. Since this study measured both parameters concurrently, the snapshot may not fully capture the time-dependent molecular-to-physiologic relationship.

The interplay between decreased red cell indices and reduced EPO expression may signify erythropoietic exhaustion, where the capacity of renal cells to induce EPO synthesis is overwhelmed by repeated donation cycles. This concept aligns with the findings of Husain *et al.,* (2024), who described similar trends and suggested that prolonged hypoxia could disrupt hypoxia-responsive transcriptional pathways.

These findings emphasize that standard haemoglobin screening, though useful, is insufficient to fully assess donor suitability and recovery status. Incorporating molecular evaluation such as EPO mRNA profiling can provide earlier detection of donors at risk of anaemia, thus improving safety and sustainability of blood donation programs.

These findings underscore that frequent donors experience reduced EPO responsiveness, possibly due to adaptive molecular feedback. Similarly, the finding in this study is supported by Ogar *et al*. (2022), who reported that donors in repeated donation programs exhibited lower hematologic indices than first-time donors, even when iron supplementation was provided, underscoring that erythropoietic regulation involves more than nutritional factors

In developed countries where molecular donor monitoring has been implemented, integrating biomarkers such as EPO and ferritin has significantly reduced donor deferrals and improved recovery times. These global experiences affirm the potential benefit of adopting similar molecular surveillance measures in Nigerian donor programs.

CONCLUSION

Regular blood donation among male donors in Ibadan was associated with lower haematocrit and haemoglobin levels, suggesting early erythropoietic strain. Although EPO mRNA expression varies, its assessment provides valuable insight into compensatory mechanisms. Occasional donors had higher EPO gene expression compared to regular donors, indicating they may be well suited for blood donation due to their enhanced ability to regenerate red blood cell.

Routine haematologic screening should be complemented with molecular monitoring (EPO mRNA) for frequent donors. Policies should be designed to regulate donation frequency and improve post-donation follow-up. There should be continuous voluntary blood donation advocacy. Further studies should explore EPO mRNA kinetics and iron status among different donor categories to optimize donor management in Nigeria.

Conflict of Interest Statement:
The authors declare no conflicts of interest.

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