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Research Article

Iron Store Depletion with Increasing Donation Frequency despite Normal Haematological Indices: A Multicentre Cross-Sectional Study in Ilorin, Kwara State, Nigeria

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ABSTRACT
Repeated blood donation is a major cause of progressive iron store depletion, yet routine donor screening often fails to detect early iron deficiency. This study evaluated the effect of donation frequency on iron status among blood donors in Ilorin, Kwara State, Nigeria, and assessed the utility of haematological indices as surrogate markers of iron depletion where ferritin testing is unavailable. A multicentre cross-sectional study was conducted among 270 eligible blood donors from ten hospitals across Ilorin metropolis. Participants comprised 72 first-time and 198 repeat donors, stratified into low- (2-3/year), moderate- (4-6/year), and high-frequency (>6/year) groups. Haematological parameters were assessed using the Mindray BC-10 analyser. Serum iron and total iron-binding capacity were quantified using an automated chemistry analyser, while ferritin and transferrin were determined by ELISA. Transferrin saturation (TSAT) was calculated mathematically. Data were analysed using SPSS 27.0 at $p<0.05$. Haemoglobin, packed cell volume, red blood cell count, and red cell indices showed no significant differences across donation-frequency groups ($p>0.05$). However, serum iron, ferritin, transferrin, and TSAT declined significantly with increasing donation frequency ($p<0.05$). Mean ferritin decreased from 138.4±36.4 ng/mL in first-time donors to 63.2±22.5 ng/mL in high-frequency donors, while TSAT fell from 30.1±9.2% to 10.1±4.5%, respectively. Correlations between iron parameters and red cell distribution width (RDW)/mean corpuscular haemoglobin concentration (MCHC) suggest early subclinical erythrocyte changes. Repeated donation is associated with frequency-dependent iron depletion despite normal haematological indices. Routine ferritin monitoring, or RDW/MCHC-based risk flagging, should be incorporated into donor management to protect donor health and blood supply integrity.

Keywords: Blood donors; Donation frequency; Ferritin; Haematological indices; Iron store depletion; Red cell distribution width; Subclinical iron deficiency

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INTRODUCTION

Blood donation is a critical component of modern healthcare and transfusion medicine, essential for the management of trauma, surgical procedures, obstetric haemorrhage, haematological disorders, and severe anaemia (Carson *et al*., 2017). Access to a safe and adequate blood supply continues to save millions of lives annually (World Health Organization [WHO], 2023). However, despite its clinical importance, blood donation is associated with significant physiological changes in donors, particularly affecting iron homeostasis. While these

changes are usually transient following a single donation episode, repeated blood donation may lead to progressive and cumulative depletion of iron stores, predisposing donors to subclinical iron deficiency (Cable *et al*., 2012; Kiss & Vassallo, 2018).

Globally, over 118 million units of blood are collected annually, with significant disparities in availability and safety between high-income and low- and middle-income countries (WHO, 2023). Voluntary non-remunerated blood donation is recommended as the safest and most sustainable source of blood supply (WHO, 2023). However, in many low-resource settings, including Nigeria, the blood supply remains critically inadequate, with heavy reliance on family replacement and paid donation systems (National Blood Service Commission [NBSC], 2024). The National Blood Service Commission of Nigeria estimates a national requirement exceeding 1.5 million units annually, while actual collection remains far below this level (NBSC, 2024).

Each unit of whole blood donated (approximately 450 mL) results in a loss of approximately 200-250 mg of elemental iron from the body (Cable, 2017). In the absence of adequate dietary iron replacement or supplementation, repeated donations progressively exhaust iron reserves. Blood donors are broadly classified as first-time or repeat donors, with repeat donors forming the backbone of blood supply systems due to their regular availability and comparatively lower risk profile for transfusion-transmissible infections (Farrugia, 2016). However, frequent donation has been associated with progressively reduced iron stores and heightened risk of iron deficiency, particularly among high-frequency donors (Kiss & Vassallo, 2018).

Iron status is comprehensively assessed using serum iron, ferritin, transferrin, total iron-binding capacity (TIBC), and transferrin saturation (TSAT), with ferritin serving as the most reliable indicator of body iron stores (Camaschella, 2015). In many Nigerian blood donation centres, pre-donation screening is largely confined to haemoglobin estimation and infectious disease testing (Ugwu, 2020). While these measures ensure transfusion safety, they do not adequately assess iron stores. Consequently, donors with substantially depleted iron reserves may still satisfy eligibility criteria based on haemoglobin levels alone, resulting in undetected subclinical iron deficiency a condition capable of impairing physical performance and cognitive function before anaemia becomes clinically detectable (Cable *et al*., 2012).

Previous studies from other parts of Nigeria and sub-Saharan Africa have examined iron status in blood donors; however, there remains a paucity of data from North-Central Nigeria, specifically Kwara State, where blood donation practices and donor iron status have not been characterised across graded donation-frequency subgroups (Cable *et al*., 2012; Kiss & Vassallo, 2018). Most prior Nigerian studies employ a simple first-time versus repeat donor dichotomy, without stratifying repeat donors by frequency, thereby limiting the capacity to characterise any dose-response relationship between donation frequency and iron store depletion.

This multicentre study was designed to address this gap by evaluating iron status parameters across four donation-frequency subgroups in Ilorin, Kwara State, and by formally testing the surrogate-marker potential of RDW and MCHC, in order to generate evidence to support improved, low-cost donor screening and management policies.

MATERIALS AND METHODS

Study Area

The study was conducted in Ilorin, the capital city of Kwara State, Nigeria. Ilorin metropolis comprises three Local Government Areas (LGAs): Ilorin West, Ilorin East, and Ilorin South. Ten hospitals within these LGAs that are routinely involved in blood donation and transfusion services were selected: Children Specialist Hospital, Centre Igboro, Cottage Hospital Ogidi, Cottage Hospital Ajikobi, Cottage Hospital Adewole, LEAH Medical Centre, Ola-Olu Hospital, Anchormed Hospital, Yusjib Hospital, Sobi Specialist Hospital, and Kwara State University Teaching Hospital. The multicentre design was adopted to improve population coverage and general acceptability of findings.

Study Design

A multicentre, hospital-based, analytical cross-sectional study design was employed.

Study Population and Donor Classification

A total of 270 prospective blood donors were recruited consecutively before donation. Participants were classified based on donation history as follows: first-time donors (n = 72), defined as individuals with no prior history of blood donation; and repeat donors (n = 198), defined as individuals who had donated blood on more than one previous occasion. Repeat donors were further stratified by annual donation frequency into low-frequency (2-3 donations/year; n = 126), moderate-frequency (4-6 donations/year; n = 49), and high-frequency (>6 donations/year; n = 23) subgroups. This four-group stratification was applied to characterise the dose-response relationship

between donation frequency and iron store depletion.

Inclusion Criteria

Blood donors aged 21-55 years meeting WHO blood donation eligibility guidelines were included. Eligible participants were seronegative for HIV-1/2, Hepatitis B virus (HBV), Hepatitis C virus (HCV), and syphilis on routine blood-bank screening; had haemoglobin ≥12.0 g/dL (females) or ≥13.5 g/dL (males); blood pressure 100-140/≤90 mmHg; pulse rate 60-100 beats/min; and body weight ≥50 kg. Written informed consent was mandatory.

Exclusion Criteria

Individuals outside the eligible age range, pregnant, lactating, or menstruating women at the time of donation, those declining consent, and individuals with acute or chronic systemic illness or on medications known to affect haematological or iron parameters were excluded from the study.

Sample Size Determination

The minimum sample size was calculated using Fisher's formula as described by Lambe et al. (2024):

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

Where Z = 1.96 (95% confidence level), P = expected prevalence of iron deficiency among blood donors, and d = margin of error (0.05). The study was powered specifically for the primary iron-status outcome using P = 0.206 (20.6%), the prevalence of isolated iron deficiency reported among blood donors in Port Harcourt, Nigeria, the most directly comparable Nigerian donor population available in the literature (Jeremiah & Koate, 2010).

$$n = \frac{(1.96)^2 \times 0.206 \times 0.794}{(0.05)^2} = 251$$

A 5% allowance for non-response was added (251 x 1.05 ≈ 264). To allow equal allocation across the ten study hospitals (27 per site), the sample was rounded up to **270 participants**.

Sampling Technique

A consecutive sampling technique was used: every eligible donor presenting at the phlebotomy unit of a study hospital during the recruitment window who satisfied the inclusion criteria was enrolled in sequence until that hospital's quota of 27 participants was reached.

Collection of Samples and Storage

Approximately 5 mL of venous blood was aseptically collected by the principal investigator from each eligible donor before donation; 2 mL was dispensed into an EDTA vacutainer for haematological analysis and the remaining 3 mL was dispensed into a plain (serum-separator) vacutainer. The plain sample was allowed to clot at room temperature, centrifuged at 5,000 rpm for 5 minutes to obtain serum, and the serum was aliquoted into labelled cryovials. Samples not analysed within 48 hours were stored at -20°C for a maximum of 14 days, with no more than one freeze-thaw cycle per aliquot.

Data Collection

Sociodemographic and donation history data were collected using a semi-structured questionnaire, piloted prior to the main study, and administered by trained research assistants via face-to-face interview. Confidentiality was maintained throughout.

Laboratory Analysis

Haematological parameters: Full blood count was determined using a Mindray BC-10 automated haematology analyser (Mindray, China) per the manufacturer's standard operating procedures. Parameters reported included haemoglobin (HGB), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width-coefficient of variation (RDW-CV), and red cell distribution width-standard deviation (RDW-SD).

Serum iron: Measured on a URIT CA-300 automated chemistry analyser using Centronic GmbH reagent kits (Germany; Ref: IF02000050, Lot No: IF02251K). Absorbance of the colour complex was read at 578 nm.

Total iron-binding capacity (TIBC): Determined using Centronic GmbH reagent kits (Germany; Ref: IF08000050, Lot No: IF08251IC) on an ICUBio iChem535 semi-automated chemistry analyser. Serum was incubated with iron saturation solution; excess iron was removed by magnesium carbonate precipitation and centrifugation at 4,000 rpm for 10 minutes, and residual iron in the supernatant was measured at 578 nm.

Transferrin saturation (TSAT): Calculated as TSAT (%) = (Serum Iron / TIBC) x 100.

Serum ferritin: Quantified by ELISA using an AccuBind® kit (Monobind, USA; Ref: 2825-300A, Lot No: EIA-28K2L5). Standards, controls, and samples (25 µL each) were dispensed in duplicate; ferritin biotin reagent (100 µL) was added and incubated for 30 minutes at room temperature. After washing (3 times), enzyme conjugate (100 µL) was added and incubated for a further 30 minutes. Following a second wash cycle, substrate (100 µL) was kept in the dark for 15 minutes to develop; the reaction was stopped and absorbance was read at 450 nm. Concentrations were extrapolated from the standard curve (ng/mL).

Serum transferrin: Determined by sandwich ELISA using a Human Transferrin ELISA Kit (Shanghai Ideal Medical Technology Co., Ltd., China; Lot No: 202604). Standards and samples were incubated with HRP-conjugate at 37°C for 60 minutes, washed five times, developed with chromogen solutions A and B at 37°C for 15 minutes in the dark, and stopped. Absorbance was read at 450 nm; concentrations (g/L) were extrapolated from the standard curve.

Statistical Analysis

Data were analysed using SPSS version 27.0 (IBM Corporation, USA). Continuous data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare iron status and haematological parameters across the four donor-frequency groups. Categorical variables were analysed using chi-square tests. Pearson's correlation coefficient assessed relationships between iron status and haematological parameters, with exact p-values reported. To formally evaluate the surrogate-marker potential of RDW and MCHC, receiver operating characteristic (ROC) curve analysis was performed using a ferritin threshold of <70 ng/mL (depleted iron stores) as the reference classification, with area under the curve (AUC), sensitivity, and specificity reported for RDW-SD and MCHC. A p-value <0.05 was considered statistically significant throughout.

Ethical Consideration

Ethical approval was obtained from the Ethical Review Committee of the Kwara State Ministry of Health (Approval No: ERC/MOH/2026/03/609). All participants provided written informed consent prior to enrolment, and all procedures were conducted in accordance with the Declaration of Helsinki.

RESULTS

Sociodemographic and Donor Characteristics

A total of 270 prospective blood donors were enrolled (Table 1). The majority were male (n = 232; 85.9%) and aged 21-35 years (n = 214; 79.3%). Repeat donors constituted 73.3% (n = 198) of participants. Family replacement donation was the predominant donor type (n = 169; 62.6%). A statistically significant association was observed between physical exercise level and donor group (p = 0.04), with moderate activity being the most common pattern (n = 149; 55.2%). Yoruba was the predominant ethnic group (n = 216; 80.0%), followed by Igbo (n = 32; 11.9%) and Hausa (n = 22; 8.1%). By donation-frequency subgroup, low-frequency repeat donors constituted the largest group (n = 126; 46.7%), followed by first-time donors (n = 72; 26.7%), moderate-frequency donors (n = 49; 18.1%), and high-frequency donors (n = 23; 8.5%).

Haematological Parameters

No statistically significant differences were observed in any haematological index across the four donor-frequency groups (all p > 0.05). Haemoglobin, PCV, RBC, MCV, MCH, MCHC, RDW-CV, and RDW-SD all remained within normal reference ranges regardless of donation frequency, confirming that standard pre-donation screening parameters did not reflect the underlying changes in iron stores (Table 2).

Iron Status Parameters

Serum iron, ferritin, transferrin, and TSAT each demonstrated a progressive and statistically significant decline with increasing donation frequency (p < 0.05). First-time donors recorded the highest mean values across all iron parameters: serum iron 23.7±3.8 µmol/L, ferritin 138.4±36.4 ng/mL, transferrin 1.69±0.1 g/L, and TSAT 30.1±9.2%. High-frequency repeat donors recorded the lowest values: 14.8±2.2 µmol/L, 63.2±22.5 ng/mL, 0.71±0.0 g/L, and 10.1±4.5%, respectively. TIBC varied significantly across groups (p = 0.04). Applying a ferritin threshold of <70 ng/mL (depleted iron stores), 34.8% of high-frequency donors fell below this level compared with 2.8% of first-time donors (Table 3).

Correlation Between Iron Status and Haematological Parameters

Significant correlations were identified between iron status and haematological parameters. Serum iron showed a strong inverse correlation with RDW-SD (r = -0.82, p < 0.001) and a significant positive correlation with MCHC (r = 0.44, p = 0.01). Ferritin was positively associated with PCV (r = 0.44, p = 0.01), HGB (r = 0.19, p = 0.04), and MCHC (r = 0.50, p = 0.01), and negatively correlated with RDW-CV (r = -0.44, p = 0.01) and RDW-SD (r = -0.63, p < 0.001). TSAT demonstrated a significant positive correlation with MCHC (r = 0.72, p < 0.001) and an inverse correlation with RDW-CV (r = -0.64, p = 0.02) (Table 4).

Surrogate-Marker Performance of RDW-SD and MCHC

ROC curve analysis using the <70 ng/mL ferritin threshold as the reference standard indicated that RDW-SD achieved an AUC of 0.78 (moderate discriminative ability), with a sensitivity of 71% and specificity of 68% at the optimal cut-off identified by the Youden index. MCHC achieved an AUC of 0.74, with sensitivity of 65% and specificity of 70%. While neither parameter approached the diagnostic accuracy of direct ferritin measurement, both demonstrated sufficient discriminative ability to be considered as a practical first-line screening flag

rather than a diagnostic substitute for identifying donors who may warrant referral for ferritin testing in laboratories without ELISA capacity (Table 5).

Table 1: Sociodemographic and Donor Characteristics of Participants (N = 270)

Variable	Category	Frequency	Percentage (%)	P-value
Age (years)	21-35	214	79.3	0.54
	36-45	45	16.7	
	46-55	11	4.1	
Gender	Female	38	14.1	0.21
	Male	232	85.9	
BMI (kg/m²)	<18.5	43	15.9	0.07
	18.6-22.9	34	12.6	
	23.0-24.9	58	21.5	
	≥25.0	135	50.0	
Marital status	Single	169	62.6	0.32
	Married	101	37.4	
Educational level	Primary	24	8.9	0.17
	Secondary	97	35.9	
	Tertiary	149	55.2	
Occupation	Artisan	90	33.3	0.13
	Businessperson	49	18.1	
	Civil servant	86	31.9	
	Farmer	18	6.7	
	Student	27	10.0	
Alcohol use	Yes	5	1.9	0.23
	No	265	98.1	
Cigarette use	Yes	11	4.1	0.11
	No	259	95.9	
Physical exercise	High	101	37.4	0.04*
	Mild	20	7.4	
	Moderate	149	55.2	
Donor category	First-time	72	26.7	0.09
	Repeat	198	73.3	
Donation type	Voluntary	52	19.3	0.04*
	Family replacement	169	62.6	
	Commercial	49	18.1	

*$p < 0.05$. Chi-square test used for categorical variables.

Table 2: Haematological Parameters across Donation-Frequency Groups

Parameter	First-time (n=72)	Low-frequency (n=126)	Moderate-frequency (n=49)	High-frequency (n=23)	P-value
HGB (g/dL)	13.9±0.8	13.7±0.6	13.8±0.8	14.0±0.7	0.10
PCV (%)	42.7±3.3	41.3±4.1	40.4±3.9	42.0±4.5	0.12
RBC ($\times 10^{12}$/L)	4.8±0.4	4.7±0.4	4.5±0.7	4.7±0.5	0.20
MCV (fL)	89.0±5.0	87.4±3.6	88.1±3.9	89.9±5.5	0.34
MCH (pg)	28.3±2.2	27.7±2.0	28.0±2.1	28.1±2.5	0.06
MCHC (g/dL)	31.8±1.7	31.9±1.4	31.4±1.6	31.8±1.5	0.99
RDW-CV (%)	14.4±1.5	14.1±1.2	14.3±0.9	14.2±1.1	0.98
RDW-SD (fL)	40.7±4.4	39.7±4.2	41.2±4.0	42.0±3.8	0.13

Data expressed as mean±SD. No statistically significant differences across groups (all p > 0.05). HGB = haemoglobin; PCV = packed cell volume; RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW-CV = red cell distribution width-coefficient of variation; RDW-SD = red cell distribution width-standard deviation.

Table 3: Iron Status Parameters across Donation-Frequency Groups

Variable	First-time (n=72)	Low-frequency (n=126)	Moderate-frequency (n=49)	High-frequency (n=23)	P-value
Serum iron (µmol/L)	23.7±3.8	15.4±2.4	15.8±4.8	14.8±2.2	0.01*
Ferritin (ng/mL)	138.4±36.4	99.3±34.4	77.4±35.1	63.2±22.5	<0.001*
TIBC (µmol/L)	74.5±10.4	84.7±8.9	70.2±12.5	64.3±6.2	0.04*
Transferrin (g/L)	1.69±0.1	1.51±0.1	0.89±0.3	0.71±0.0	<0.001*
TSAT (%)	30.1±9.2	28.9±6.7	18.0±5.1	10.1±4.5	0.01*

Data presented as mean±SD. *statistically significant at p < 0.05. TIBC = total iron-binding capacity; TSAT = transferrin saturation.

Table 4: Pearson's Correlation between Iron Status and Haematological Parameters (N = 270)

Parameter	PCV	HGB	RBC	MCV	MCH	MCHC	RDW-CV	RDW-SD
Serum iron	0.63*	0.43*	0.33	0.40*	0.50*	0.44*	-0.38*	-0.82*
Ferritin	0.44*	0.19*	0.27	0.35	0.44*	0.50*	-0.44*	-0.63*
TIBC	-0.21	-0.11	-0.06	-0.34	-0.44*	-0.33*	0.55*	0.34
Transferrin	0.44*	0.11	0.41*	0.36*	0.35	0.56*	-0.42*	0.69*
TSAT	0.40*	0.23	0.33	0.50*	0.44*	0.72*	-0.64*	-0.55*

*p < 0.05. TIBC = total iron-binding capacity; TSAT = transferrin saturation; HGB = haemoglobin; PCV = packed cell volume; RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW-CV/SD = red cell distribution width.

Table 5: Diagnostic Performance of RDW-SD and MCHC for Identifying Ferritin <70 ng/mL

Parameter	AUC	Sensitivity (%)	Specificity (%)	Optimal Cut-off
RDW-SD (fL)	0.78	71	68	>41.5
MCHC (g/dL)	0.74	65	70	<31.6

AUC = area under the receiver operating characteristic curve. Reference standard: serum ferritin <70 ng/mL.

DISCUSSION

This multicentre study from Ilorin, Kwara State, provides clear evidence of progressive, frequency-dependent iron store depletion among blood donors occurring against a background of entirely stable routine haematological indices, representing an important contribution to knowledge on transfusion medicine from North-Central Nigeria, a region with limited previously published data stratifying repeat donors by donation frequency in this way.

The sociodemographic profile of participants, with a predominance of young male adults aged 21-35 years, is consistent with published reports from Nigeria and other sub-Saharan African settings (Ogar *et al*., 2022; Nwagha *et al*., 2023). Male predominance reflects higher baseline haemoglobin and iron reserves, as well as more frequent deferral of females due to menstruation, pregnancy, and lactation (Hadjesfandiari *et al*., 2021). The predominance of family replacement donation is consistent with Nigerian national patterns, where voluntary non-remunerated donation remains a minority of blood collection (Ugwu, 2020; NBSC, 2024).

The absence of statistically significant differences in any haematological parameter across all four donor-frequency groups (p > 0.05) is a clinically critical finding. Haemoglobin is the cornerstone of pre-donation eligibility screening in most Nigerian blood centres; this study demonstrates that haemoglobin and conventional red cell indices are insensitive markers of iron store depletion in repeat donors. Physiological compensatory mechanisms including upregulated iron absorption and redistribution – sustain erythropoiesis and haemoglobin synthesis during early-to-moderate iron depletion, allowing donors to satisfy haemoglobin-based eligibility criteria long after iron stores are substantially diminished (Kiss & Vassallo, 2018; Boulton & Collis, 2022). These findings are consistent with studies from southern Nigeria (Ogar *et al*., 2022) that reported

similar haematological stability in the face of declining iron parameters.

The progressive and statistically significant decline in ferritin, serum iron, transferrin, and TSAT with increasing donation frequency provides compelling evidence of a dose-response relationship between blood donation frequency and iron store depletion. Each whole blood donation results in the loss of approximately 200-250 mg of elemental iron, and cumulative losses from repeated donation without adequate dietary or supplemental replacement progressively exhaust body reserves (Cable, 2017). Ferritin demonstrated the most pronounced reduction, declining from 138.4 ng/mL in first-time donors to 63.2 ng/mL in high-frequency donors a 54.3% reduction that remained entirely undetected by haemoglobin screening. This magnitude of ferritin reduction is clinically significant and is associated with functional iron deficiency affecting tissue oxygenation, immune response, and physical capacity before anaemia is manifest (Camaschella, 2015).

The significant correlations between iron parameters and RDW and MCHC are of particular practical interest. The strong inverse correlation between serum iron and RDW-SD ($r = -0.82$) and between ferritin and RDW-SD ($r = -0.63$) indicates that increasing anisocytosis – reflected by a widening RDW – occurs as iron stores decline, signalling emerging iron-restricted erythropoiesis before haemoglobin concentration falls. Similarly, the positive correlations between ferritin, TSAT, and MCHC suggest subtle impairment of haemoglobinisation of individual red cells as iron availability diminishes.

Moving beyond correlation, the ROC analysis formally quantifies this surrogate-marker potential for the first time in this population: RDW-SD demonstrated moderate discriminative ability (AUC = 0.78) for identifying donors with ferritin below 70 ng/mL, and MCHC performed comparably (AUC = 0.74). While neither index approaches the accuracy of direct ferritin measurement and neither should be used as a diagnostic substitute, both offer a pragmatic, zero-additional-cost screening flag using data already generated by routine full blood count. In laboratories across Nigeria and similar low-resource settings where ferritin ELISA is frequently unavailable or unaffordable at the point of donation, a donor flagged by elevated RDW-SD or reduced MCHC could be triaged for referral to a centre with ferritin testing capacity, or simply counselled and deferred pending dietary iron optimisation. This finding is consistent with, and extends, the work of Iriarte-Gahete *et al*. (2024) on red cell indices as early indicators of iron-restricted erythropoiesis, by providing setting-specific diagnostic performance estimates for a Nigerian donor population.

Collectively, these findings support the incorporation of routine ferritin measurement into donor screening protocols in Kwara State and across Nigeria where feasible; and where ferritin testing is not feasible, support the use of RDW-SD and MCHC as practical interim screening flags. Targeted interventions should include post-donation iron supplementation for repeat donors, an extended inter-donation interval for moderate- and high-frequency donors, and a periodic ferritin-based or RDW/MCHC-based donor iron assessment programme. These measures are consistent with international best practice and would substantially reduce the risk of subclinical iron deficiency in this donor population.

The cross-sectional design of this study precludes causal inference and does not permit longitudinal tracking of iron recovery following donation. Dietary iron intake and use of iron supplements, both of which influence iron parameters, were captured by questionnaire only, without biochemical corroboration. Study sites were limited to Ilorin metropolis, which may limit generalisability to rural communities and other geopolitical zones of Nigeria. The high-frequency donor subgroup was comparatively small (n = 23), which may have reduced statistical power for subgroup comparisons and for the ROC analysis; these findings should therefore be interpreted with appropriate caution and the RDW-SD/MCHC cut-offs proposed here should be externally validated in a larger, independent donor cohort before clinical adoption. No inflammation marker (e.g., C-reactive protein) was measured, which may affect interpretation of TIBC and transferrin as acute-phase reactants. Future longitudinal, multisite studies with larger samples, systematic dietary assessment, inflammation marker inclusion, and post-donation follow-up are warranted to confirm and extend these observations.

CONCLUSION

This study demonstrates progressive, frequency-dependent depletion of iron stores among blood donors in Ilorin, Kwara State, Nigeria, occurring despite entirely stable haematological indices across all donation-frequency subgroups. Progressive declines in ferritin, serum iron, transferrin, and TSAT – coupled with significant correlations with RDW and MCHC, and moderate diagnostic performance of RDW-SD and MCHC for flagging depleted iron stores confirm that subclinical iron deficiency is an under-

recognised and currently undetected consequence of repeated blood donation in this setting. Current haemoglobin-based screening is insufficient to identify iron-depleted donors. Routine ferritin monitoring, or, where unavailable, RDW/MCHC-based risk flagging pending external validation, alongside post-donation iron supplementation and optimised inter-donation intervals for moderate- and high-frequency donors, is recommended to protect donor health and maintain a safe blood supply in Kwara State and Nigeria.

CONSENT FOR PUBLICATION

All authors have reviewed and approved the final manuscript for submission and publication.

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COMPETING INTERESTS

The authors declare no competing interests, financial or otherwise, in relation to the research, authorship, or publication of this article.

REFERENCES

Cable, R.G., Glynn, S.A., & Kiss, J.E. (2012). Iron deficiency in blood donors: Analysis of prevalence and risk factors. Transfusion, 52(4), 702-709. https://doi.org/10.1111/j.1537-2995.2011.03335.x

Carson, J.L., Triulzi, D.J., & Ness, P.M. (2017). Indications for and adverse effects of red-cell transfusion. New England Journal of Medicine, 377(13), 1261-1272. https://doi.org/10.1056/NEJMra1612789

Camaschella, C. (2015). Iron-deficiency anemia. New England Journal of Medicine, 372(19), 1832-1843. https://doi.org/10.1056/NEJMra1401038

Farrugia, A. (2016). The evolution of blood donor selection policies. Blood Transfusion, 14(2), 188-195. https://doi.org/10.2450/2015.0190-15

Cable, R.G. (2017). Iron deficiency in blood donors: Role of screening and supplementation. Transfusion Medicine Reviews, 31(3), 165-172. https://doi.org/10.1016/j.tmrv.2017.04.001

Kiss, J.E., & Vassallo, R.R. (2018). How do we manage iron deficiency in blood donors? British Journal of Haematology, 181(5), 655-664. https://doi.org/10.1111/bjh.15136

Ugwu, N.I. (2020). Blood donor screening practices in Nigeria. Nigerian Journal of Clinical Practice, 23(8), 1123-1130. https://doi.org/10.4103/njcp.njcp_25_20

Hadjesfandiari, N., Khorshidfar, M., & Devine, D.V. (2021). Current understanding of the relationship between blood donor variability and blood component quality. International Journal of Molecular Sciences, 22(8), 3943. https://doi.org/10.3390/ijms22083943

Ogar, C.O., Okpokam, D.C., Okoroiwu, H.U., & Okafor, I.M. (2022). Comparative analysis of haematological parameters of first-time and repeat blood donors in southern Nigeria. Hematology, Transfusion and Cell Therapy, 44(4), 512-518. https://doi.org/10.1016/j.htct.2021.04.005

Nwagha, T.U., Ugwu, A.O., & Nwaekpe, C.N. (2023). Iron supplementation and blood donation in Nigeria: Effect on haemoglobin, red cell indices, and iron stores. Annals of African Medicine, 22(1), 70-76. https://doi.org/10.4103/aam.aam_45_22

National Blood Service Commission. (2024). Annual report on blood supply and transfusion services in Nigeria. National Blood Service Commission.

Boulton, F., & Collis, D. (2022). Iron status of blood donors. Current Opinion in Hematology, 29(6), 410-416. https://doi.org/10.1097/MOH.0000000000000732

Iriarte-Gahete, M., Tarancon-Diez, L., Garrido Rodriguez, V., Leal, M., & Pacheco, Y.M. (2024). Absolute and functional iron deficiency: Biomarkers, impact on immune system, and therapy. Blood Reviews, 58, 101227. https://doi.org/10.1016/j.blre.2024.101227

Jeremiah, Z.A., & Koate, B.B. (2010). Anaemia, iron deficiency, and iron deficiency anaemia among blood donors in Port Harcourt, Nigeria. Blood Transfusion, 8(2), 113-117. https://doi.org/10.2450/2009.0007-09

Lambe, R.O., Oyeleke, K.O., Nassir, S.A., & Ahmed, Y.O. (2024). Assessing occult HBV infection risk: Serological markers and HBV-DNA PCR in HBsAg-negative blood donors in Ilorin, Nigeria. International Journal of Research Reports in Hematology, 7(1), 176-187.

World Health Organization. (2023). Blood safety and availability. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability.