Original Article

A Comparative Study of Serum Ferritin Levels among Unfit and Fit Blood Donors

Olusola Festus Olowoselu[1], Ebele Uche[2], Olufemi Oyedeji[1], Oluwakemi E. Otokiti[3], Oluwamuyiwa Anthony Ayanshina[4], Akinsegun Akinbami[2], Vincent Osunkalu[1]

[1]Department of Haematology and Blood Transfusion, Faculty of Clinical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria, [2]Department of Hematology and Blood Transfusion, Lagos State University, College of Medicine, Lagos, Nigeria, [3]Department of Haematology and Blood Transfusion, Lagos University Teaching Hospital, Lagos, Nigeria, [4]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria

Abstract

Background: Cheap methodologies are being utilized by low-resource countries to determine blood donors' fitness. Important hematological biomarkers might have to be evaluated to enhance the use of these methods. **Aims:** The study evaluated the pattern of serum ferritin in 18–24 fit and unfit prospective blood donors (PDBs) and the prevalence of iron store deficiency. **Settings and Design:** This study was a cross-sectional, comparative study which was conducted at the blood donor clinic of the Lagos University Teaching Hospital. **Materials and Methods:** Blood samples were collected by venipuncture into sodium-ethylenediaminetetraacetic acid and plain bottles. The latter was centrifuged and used for ferritin determination via human ferritin enzyme-linked immunosorbent assay test kit, while the former was used for red cell indices analysis using an autoanalyzer. **Statistical Analysis:** Data were analyzed using SPSS version 20, values were presented as mean ± standard deviation, and $P \leq 0.05$ was considered statistically significant. **Results:** A total of 263 PDB were recruited into the study consisting of 210 (79%) males and 53 (21%) females, with a mean age of 32.88 ± 8.22. Only 110 (41.8%) of the participants were considered fit, while 153 (58.2%) were unfit using copper sulfate specific gravity. There was no statistically significant difference ($P = 0.301$) in the mean level of serum ferritin in unfit blood donors (74.5 ± 90.8 μg/L) compared to that of the fit blood donors (61.5 ± 54.5 μg/L). The prevalence of iron store depletion among blood donors in Lagos state was 11.8% (31 of 263) with a higher proportion (7.6%) occurring among unfit donors. However, low levels of serum ferritin (<15 μg/L) were significantly associated with the occurrence of anemia (hemoglobin < 12.5 g/gl) among unit donors (19%; $P = 0.05$). **Conclusion:** Although serum ferritin depletion appears to be higher in the unfit blood donors, the use of serum ferritin as an index for the screening and determination of PDBs' fitness requires further evaluation.

Keywords: Blood donors, copper sulfate specific gravity method, hemoglobin, serum ferritin

Introduction

Iron is essential for optimal human physiological function; however, its role as a determinant of blood donation fitness needs to be critically examined since it is not one of the routinely considered prerequisites for blood donation fitness as currently practiced in most centers.[1] However, hemoglobin (Hb) level is primarily used in the assessment of blood donors' fitness,[2] and the prerequisite level is set at ≥12.5 g/dL before clearance is given for possible blood donation deposition.[3]

Deferral of blood donors as a consequence of factors including anemia, which is an indication of low Hb, mostly upset donors and often causes loss of donors permanently.[4,5] Several methods including HemoCue and Salhi methods and copper sulfate ($CuSO_4$) specific gravity method have been employed in the estimation of Hb and have been used for classifying prospective blood donors (PDBs) as unfit or fit.[6,7] The shortcomings of $CuSO_4$ specific gravity method to screen blood donor for eligibility have been documented in our previous study.[8] In addition, it is noteworthy to mention that there are other possible causes of anemia, one of which is hinged on iron deficiency.[9,10] An estimated 0.5 mg of iron is reportedly lost per 1 ml volume of blood donated.[3] Therefore,

Access this article online

Quick Response Code:

Website: www.nigeriamedj.com

DOI: 10.4103/nmj.NMJ_48_19

Address for correspondence: Dr. Olusola Festus Olowoselu, Department of Haematology and Blood Transfusion, Faculty of Clinical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria.
E-mail: solifes@yahoo.com

Submitted: 30-Mar-2019 **Revised:** 29-Sep-2019
Accepted: 09-Dec-2019 **Published:** 24-Feb-2020

How to cite this article: Olowoselu OF, Uche E, Oyedeji O, Otokiti OE, Ayanshina OA, Akinbami A, *et al*. A comparative study of serum ferritin levels among unfit and fit blood donors. Niger Med J 2019;60:312-6.

early information about the iron status of blood donors might annul this disappointment and enhance continuous and regular blood banking.[11]

Erhabor *et al*.[3] reported the prevalence of iron deficiency among blood donors geographically found in Sokoto, Northwestern Nigeria. Vijatha *et al*.[12] reported that iron stores in the body are quite small and the depletion of iron is commonly found among blood donors. Despite normal Hb level and no symptoms of anemia, there might be a possibility of low iron levels often confirmed by a low blood serum ferritin.[13] Serum ferritin preferably is the serologic marker of iron stores.[14] Thus, evaluating the iron store status alongside Hb, determination of blood donors might be crucial to ensuring blood donor safety.

Therefore, we hypothesized that since low serum ferritin levels have been associated with blood donors, there might be a possibility whereby iron status determination may augment the affordable $CuSO_4$ specific gravity method in order to determine blood donors' fitness. In as much as the physiological importance of iron cannot be overemphasized, it is important to discern its role in the determination of the fate of PDBs. Hence, this present study evaluated the influence of iron store on the fitness of blood donors as well as depicted the prevalence of iron store depletion in blood donors.

Materials and Methods

Study design

This was a cross-sectional, comparative study between PDBs considered fit and those unfit using $CuSO_4$ specific gravity test. It was conducted at the blood donor clinic of the Lagos University Teaching Hospital between March and April 2012 after ethical approval (ADM/DCST/HREC/285) reference number was obtained from the hospital ethical committee. All participants were first-time donors

Study population and sampling technique

PDBs ($n = 263$) who were classified into fit and unfit based on the $CuSO_4$ specific gravity test were recruited consecutively for this study. There were 153 unfit blood donors and 110 fit blood donors. Having sought participants' informed consent and met the inclusion criteria, a structured questionnaire was administered to each prospective participant to obtain information on demography, health status, dietary history, menstrual pattern, exercise, and social behavioral history.

The inclusion criteria for this study included all consenting healthy adults aged 18–60 years, weighing ≥50 kg and, having given informed consent. Exclusion criteria were the donors who weigh <50 kg, anemic menstruating women, and all persons with hemolytic anemia, for example, sickle cell disease. All persons with transfusion transmissible infections such as HIV, viral hepatitis, and syphilis; all persons with medical conditions such as cardiovascular diseases or had major surgery in the past 1 year; and All persons with social behaviors such as homosexuality, intravenous drug users, and body piercing/tattooing were also excluded. All pregnant women or <6 weeks postpartum, all persons on medications such as aspirin and finasteride, and all persons having a history of previous blood transfusion, especially in the past 3 months, were also excluded.

Specimen collection and preparation

Ten milliliters of blood was collected from each of the study participants by venipuncture. Five milliliters (5 ml) of the blood collected was dispensed into sodium ethylenediaminetetraacetic acid specimen bottle and was subsequently used for the estimation of red cell indices within 2 h of collection using the Hematology Autoanalyzer (Sysmex KX21® Japan). Meanwhile, the remaining 5 ml of blood was transferred to plain disposable plastic tubes and was allowed to stand at room temperature to clot. This was centrifuged, serum was transferred into plain cryotube, stored at −80°C, and used for the estimation of serum ferritin.

Serum ferritin determination

The level of serum ferritin was quantified using Human Ferritin Enzyme-linked Immunosorbent Assay (ELISA) Test Kit (Diagnostic Automation Inc., USA) based on a solid-phase ELISA. The assay system utilizes an anti-ferritin antibody (microtiter well) immobilization and another mouse monoclonal anti-ferritin antibody as the antibody-enzyme (horseradish peroxidase) conjugate solution. Briefly, 20 µl of standard, test, and control samples were dispensed into appropriate wells followed by the addition of 100 µl of enzyme conjugate reagent into each well. This mixture was incubated at room temperature for 60 min. The incubated microliter wells were rinsed, flicked 5 times with washing buffer, the wells were sharply struck onto paper towels to remove all residual water droplets, and were further incubated at room temperature in the dark for 20 min. The reaction was stopped by adding 100 µl of stop solution to each well, gently mixed for 30 s, and the optical density (OD) was read at 450 nm with a microliter reader within 30 min. By plotting the OD on y-axis against the standard concentration on x-axis, the concentrations of each test and control sample were extrapolated.

Statistical analysis

Data was analysed using SPSS version 16.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The values were expressed as mean ± standard error of mean. Comparison between the mean values was done using the nonparametric Student's *t*-test. The cutoff value for normal Hb level was ≥12.5 g/dl,[2,3,8] while that of serum ferritin was ≥15 µg/L.[15] The unfit and fit blood donors groups were subgrouped as anemia (<12.5 g/dl) and normal (≥12.5 g/dl) based on the Hb level estimation by an autoanalyzer and as abnormal (<15 µg/L) and normal (≥15 µg/L) based on the serum ferritin level estimation. These subgroupings were used for cross-tabs analysis. (N. B.: Unfit PDBs with normal Hb estimation were taken to be falsely deferred by the $CuSO_4$ specific gravity method, while fit PDBs with anemia Hb estimation were taken to be falsely passed). Comparison between Hb levels and serum ferritin was done

using the Fischer's test, and $P \leq 0.05$ was considered statistically significant.

Results

A total of two hundred and sixty-three PDBs were recruited consisting 210 males and 53 females. The mean age of all participants was 32.88 ± 8.22 years. There was no statistically significant difference (P = 0.301) in the mean level of serum ferritin in unfit blood donors (74.5 ± 90.8 μg/L) as compared to that of the fit blood donors (61.5 ± 54.5 μg/L) [Table 1]. However, the mean ferritin level of fit male blood donors (61.94 + 54.06 μg/L) was significantly (P = 0.04) lower than that of unfit male blood donors (88.11 + 99.28 μg/L). In contrast, the level of ferritin of fit female blood donors (57.17 + 63.86 μg/L) was not significantly (P = 0.39) higher than ferritin level in unfit female blood donors (38.38 + 47.93 μg/L) [Table 2]. About 8.7% (8 of 91) of the fit PDBs (with >12.5 g/dl Hb level) and 19.3% (12 of 62) of the unfit PDBs (with <12.5 g/dl Hb level) had abnormal levels of serum ferritin [Table 3].

Fifty (80.7%) of the total unfit blood donors who have been classified to be anemic (n = 62) based on their Hb level (<12.5 g/dl) as estimated by an autoanalyzer were found to have normal levels of serum ferritin (≥15 μg/L), thus showing significant association between serum ferritin and Hb among unfit donors (χ^2 = 3.621; P = 0.05). Conversely, none of the fit donors (as identified by the $CuSO_4$ test) whose Hb was <12.5 g/dl had low serum ferritin even though the association between low serum ferritin and anemia did not attain statistical significance (χ^2 = 1.333; P = 0.248). Of the unfit donors classified by $CuSO_4$ test (153 of 263), 59.5% (91 of 153) were falsely deferred unfit blood donors. However, low serum ferritin (<15 μg/L) level was observed in 8.7% (8 of 91) of these falsely deferred blood donors. Of the 62 appropriately deferred donors (40.5%), however, low serum ferritin was observed in 19.3% (12 of 62), thus showing a significant association between donor fitness and serum ferritin levels among unfit donors (χ^2 = 3.621; P = 0.05). As shown in Table 3, a total number of 18 participants of the 117 in the fit PDB category (15.4%) were falsely passed as fit. However, none of these categories of patients had low serum ferritin levels, though this association did not attain statistical significance (χ^2 = 1.333; P = 0.248) [Table 3]. The Pearson correlation coefficient in Table 4 showed a positive but insignificant correlation between the levels of serum ferritin and other red cell indices.

Table 1: Serum ferritin level in fit and unfit first-time donors

Participants (first-time donors)	*n*	Mean serum ferritin (μg/L)	*P*
PBD status			
Unfit	153	74.50±90.80	0.301
Fit	110	61.50±54.50	

Significant (P<0.05). PDB – Prospective blood donor

Table 2: Comparison of serum ferritin level between fit and unfit donors classified by sex

Participants (first time donors)	PBD status	*n*	Ferritin (μg/L)	*P*
Sex				
Male	Unfit	111	88.11±99.28	0.04**
	Fit	99	61.94±54.06	
Female	Unfit	42	38.38±47.93	0.39
	Fit	11	57.17±63.86	

**Significant (P<0.05). PDB – Prospective blood donor

Discussion

Hb level estimation is the prerequisite biomarker utilized for blood donors' fitness determination. However, in the low-resource economy, alternatively, a cheap methodology is being applied in the determination of the fitness of blood donors.[16] Our previous study has shown the use of $CuSO_4$ specific gravity method to determine Hb concentration, and the overall blood donors' fitness is not totally dependable; however, it slightly holds some promise.[8] In this study, the observed statistically nonsignificant (P > 0.05) difference in serum ferritin level in the unfit blood donors as compared to the fit blood donors suggests that the category of blood donors' fitness (fit/unfit) might not have any modulating effect on serum ferritin level. It might be that multiple factors including diet, blood donation interval, and gender may have impacted the blood donor fitness phenomenon.[1]

The observed abnormal level of serum ferritin among correctly rejected unfit blood donors and falsely rejected unfit blood donors (who are fit on the basis of Hb estimation by an autoanalyzer) is indicative of an iron store depletion

Table 3: Comparison of ferritin levels in anemic and nonanemic subjects

Participants	Ferritin	Hemoglobin		χ^2	*P*
		Anaemia (<12.5 g/dl) (%)	Normal (≥12.5 g/dl) (%)		
PBD status					
Unfit	Abnormal (<15 μg/L)	12 (19.3)	8 (8.7)	3.621	0.05**
	Normal (≥15 μg/L)	50 (80.7)	83 (91.3)		
Fit	Abnormal (<15 μg/L)	0 (0.0)	11 (12)	1.333	0.248
	Normal (≥15 μg/L)	18 (100.0)	81 (88)		

**Significant (P<0.05). PDB – Prospective blood donor

Table 4: Correlation of serum ferritin with some hematological indices

Correlation	Pearson correlation	*P*
Ferritin and Hemoglobin		
Total group=263	0.033	0.627
Unfit=153	0.045	0.584
Fit=110	0.116	0.378
Ferritin and PCV		
Total group=263	0.031	0.652
Unfit=153	0.064	0.434
Fit=110	0.089	0.507
Ferritin and MCV		
Total group=263	0.021	0.766
Unfit=153	0.029	0.722
Fit=110	0.042	0.756
Ferritin and MCH		
Total group=263	0.021	0.766
Unfit=153	0.061	0.454
Fit=110	0.042	0.756
Ferritin and MCHC		
Total group=263	0.095	0.169
Unfit=153	0.074	0.363
Fit=110	0.169	0.205

**Significant ($P<0.05$). PCV – Packed cell volume; MCV – Mean Cell Volume; MCH – Mean cell hemoglobin; MCHC – Mean cell hemoglobin concentration

occurrence among individuals irrespective of the category of blood fitness (fit/unfit). In addition, it is remarkable to note that the prevalence of iron store depletion among blood donors in this study is higher among unfit blood donors (19.3%) in comparison to fit blood donors (8.7%). Our finding corroborates the studies of Buhari *et al*.[17] and Jeremiah and Koate[18] who reported 10% and 12% as the prevalence of iron deficiency anemia among blood donors in Sokoto and Rivers States, Nigeria, respectively. Although both previous studies focused on the frequency of blood donation among participants, this present study focused on the fitness of blood donors.

Comparatively, the similar statistically significant ($P < 0.05$) prevalence of normal serum ferritin (≥15 μg/L) among the correctly deferred unfit blood donors (anemic) and the falsely deferred blood donors whose Hb level estimation was authenticated to be normal by an autoanalyzer, further supports the suggestion that blood donation may not be strongly involved or might not affect the iron store. Cable *et al*.[19,20] in a study which was done over a 2 year period also found iron deficiency anemia or absence of iron stores as measured on the basis of serum ferritin in first-time and reactivated blood donors; however, it was also reported that frequent blood donors are presented more with iron deficiency anemia or absence of iron stores. It would be recalled that statistically nonsignificant ($P > 0.05$)-positive association noted between some hematological parameters and serum ferritin level, however, means iron concentration might not be central to the buildup of red cell indices. This finding is in agreement with the report of Goldman[1] who explained that the level of Hb poorly predicts iron status.

Putting together all the evidences including the findings of this study, iron store depletion cuts across first-time, reactivated, fit and unfit blood donors. This implies that other factors might be invariably responsible for this outcome (blood donor fitness). Even though some individuals with conditions which are known to modulate serum ferritin levels have been earlier excluded from the study, there might remain some factors affecting serum ferritin that cannot be exclusively adjusted. Participants' nutritional imbalances and gender status are very strong examples of these factors. Although iron absorption in regular blood donors is reportedly higher than individuals who do not donate blood, the iron absorption does not entirely compensate for the iron loss due to increased frequency of blood donating.[21] This might be responsible for the increased prevalence of iron store depletion in unfit blood donors as compared to fit blood donors. Since iron store depletion is found in fit and unfit blood donors and the increased rate of regular and/or frequent blood donation is usually one of the major reasons for blood donor deferral, iron depletion might not largely influence the unfitness of blood donor

Conclusion

The prevalence of iron deficiency anemia among blood donors in Lagos, Nigeria, is 8.7%–19.3%, and it is higher among unfit blood donors. Serum ferritin is not a suggestible index to be considered one of the determinants that can be used for the screening and determination of PDBs' fitness. This is yet a preliminary finding; thus, it is advised that factors including blood donation interval and increased participants nationwide should be considered to have a robust conclusion. Finally, a cohort study involving iron supplementation to control nutritional imbalances among participants is recommended to have an unequivocal outlook of the level of serum ferritin in blood donor fitness.

Study limitations

Since serum ferritin is an acute-phase reactant; elimination of all factors that may affect the serum ferritin level is difficult to evaluate in this study. Other indices of iron stores were not considered in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Goldman M. Iron depletion and routine ferritin measurement in blood donors. Int Soc Blood Transfus 2015;1:124-8.
2. Shahshahani HJ, Meraat N, Mansouri F. Evaluation of the validity of a rapid method for measuring high and low haemoglobin levels in whole blood donors. Blood Transfus 2013;11:385-90.
3. Erhabor O, Imrana S, Buhari HA, Abdulrahaman Y, Wase AA, Ikhuenbor D. *et al*. Iron deficiency among blood donors in Sokoto, North Western, Nigeria. Open J Blood Dis 2014;4:33-42.
4. Bianco C, Brittenham G, Gilcher RO, Gordeuk VR, Kushner JP,

Sayers M, *et al*. Maintaining iron balance in women blood donors of childbearing age: Summary of a workshop. Transfusion 2002;42:798-805.
5. Newman BH, Newman DT, Ahmad R, Roth AJ. The effect of whole-blood donor adverse events on blood donor return rates. Transfusion 2006;46:1374-9.
6. Cable RG. Hemoglobin determination in blood donors. Transfus Med Rev 1995;9:131-44.
7. Srivastava T, Negandhi H, Neogi SB, Sharma J, Saxena R. Methods for hemoglobin estimation: A review of "what works". J Hematol Transfus 2014;2:1028.
8. Olowoselu FO, Akanmu AS, Olowoselu OI, Ayorinde AA. Sensitivity and specificity of copper sulphate test in determining fitness of blood donors. Nig Q J Hosp Med 2016;26:343-6.
9. Sagir GA, Modu BK. Morphological classification of anaemia among first-timeblood donors in Northwest Nigeria and the implication of empirical haematinics therapy. Int J Biomed Health Sci 2011;7:125-9.
10. Moreno Chulilla JA, Romero Colás MS, Gutiérrez Martín M. Classification of anemia for gastroenterologists. World J Gastroenterol 2009;15:4627-37.
11. O'Meara A, Infanti L, Stebler C, Ruesch M, Sigle JP, Stern M, *et al*. The value of routine ferritin measurement in blood donors. Transfusion 2011;51:2183-8.
12. Thomas V, Mithrason A, Silambanan S. A study to assess the iron stores of regular blood donors. Int J Clinic Biochem Res 2016;3:466-8.
13. Werbach M. Infertility Textbook of Nutritional Medicine. USA: Third Line Press, Inc.; 1999. p. 460-8.
14. Dasher K, Worthington M. Iron: Not too much and not too little. Nutrition Issues in Gastroenterology 2009;70:16-26.
15. World Health Organization. Serum Ferritin Concentrations for the Assessment of Iron Status and Iron Deficiency in Populations. Vitamin and Mineral Nutrition Information System. Geneva: World Health Organization, 2011. Available from: http://www.who.int/vmnis/indicators/serum_ferritin.pdf. [Last accessed on 2019 Mar 29].
16. James V, Jones KF, Turner EM, Sokol RJ. Statistical analysis of inappropriate results from current Hb screening methods for blood donors. Transfusion 2003;43:400-4.
17. Buhari HA, Erhabor O, Imrana S, Abubakar W, Onuigwe F, Okwesili A, *et al*. Prevalence of iron deficiency anaemia among blood donors in Sokoto, North Western, Nigeria. J Coast Life Med 2015;3:312-6.
18. Jeremiah ZA, Koate BB. Anaemia, iron deficiency and iron deficiency anaemia among blood donors in Port Harcourt, Nigeria. Blood Transfus 2010;8:113-7.
19. Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, *et al*. Iron deficiency in blood donors: Analysis of enrollment data from the REDS-II donor iron status evaluation (RISE) study. Transfusion 2011;51:511-22.
20. Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, *et al*. Iron deficiency in blood donors: The REDS-II donor iron status evaluation (RISE) study. Transfusion 2012;52:702-11.
21. Tailor HJ, Patel PR, Pandya AN, Mangukiya S. Study of various hematological parameters and iron status among voluntary blood donors. Int J Med Public Health 2017;7:61-5.