Research

Pre- and post-donation ferritin level of blood donors attending University of Calabar Teaching Hospital, Donor Clinic in Southern Nigeria

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Abstract

Background: While trying to save the patient via blood transfusion, the safety of the blood donor is paramount. This study evaluated the pre- and post- donation ferritin and packed cell volume (PCV) of donors attending University of Calabar Teaching Hospital.

Method: The study adopted descriptive longitudinal approach. A total of 18 donors with age range of 18 – 48 years were enrolled and followed up for 30 days post- donation. The serum ferritin was analyzed using ELISA method while the PCV was analyzed using the microhematocrit method. Difference between means was performed using repeated measure ANOVA while post hoc was done using Bonferroni adjustment. Prediction of return to baseline values were performed using logistic regression. Alpha value was placed at 0.05.

Results: There was a decline in ferritin and packed cell volume from pre- to post-donation. The decline in ferritin was imminent until day 14 when recovery was initiated. Significant difference was observed between the pre-donation ferritin and the rest of the days except day 30. There was also a decline in PCV from pre-donation all through with recovery noticeable after day 7. The PCV of the pre-donation was only comparable to the day 30 post-donation. Approximately 5.6% (n=1) of the subjects was iron deficient pre-donation. Approximately 25% (n=4) of the subject have returned to baseline PCV while 0% of the subjects have returned to baseline ferritin at day 30 post-donation.

Conclusion: For the safety of the donor, donation interval should be widened, and iron supplement followed up.

Keywords: Blood donation; ferritin; iron deficiency anaemia; pre-donation; post-donation

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Introduction

Blood transfusion has established itself as an integral part of medical and surgical patient management in the healthcare system in virtually every country.[1,2] The central goal of blood transfusion and donor selection is designed to ensure that the donation does not cause harm to the recipient, consequently, providing safe blood that causes no harm to the patient.[3,4] Blood donors are generally allowed to donate blood every three months for male blood donors and every four months for female blood donors according to World Health Organization.[5] Their age should be between 18 to 65 years, the weight, at least 45 kg to donate 350 ml ±10% of blood and 50 kg to donate 450 ml ±10% of blood. More so, they are expected to be generally healthy according to history obtained during pre-donation counselling.[6,7]

Blood transfusion centers routinely screen prospective donors for transfusion transmissible infectious, haemoglobin or packed cell volume and other reasons for deferrals.[8,9,10] The sole haematological screening in Nigeria is haemoglobin or packed cell volume. Despite all these safeguard measures, investigations on donors can have tremendous immediate complication on body's iron balance, which is an important safety issue.[11,12] A complete single adult pack donation (400 – 500 ml) eliminates about 250 mg of iron, which translates to about 4 – 10% of the total body iron stores.[12] Following continuous depletion of iron stores, the body adapts to reduced concentration of iron or develops iron deficiency.[12,13] The development of iron deficiency involves three sequential events: iron depletion, iron deficient erythropoiesis and finally iron deficiency anaemia.[3,14,15]

To evaluate iron stores, various methods such as ferritin, zinc protoporphyrin (ZPP) and soluble transferring receptor has been employed.[16,17] Ferritin is a storage protein that is found predominantly in the liver, spleen, and bone marrow. Iron is stored primarily in the body in the form of ferritin. Small amount of ferritin is found in plasma and the concentration of which correlates positively with the size of the total body iron stores in the absence of inflammation.[16] A low serum ferritin value reflects depleted iron stores. It is stable and not affected by recent ingestion of iron and appears to reflect the iron stores accurately and quantitively.[17]

Since haemoglobin/hematocrit levels may be normal in the presence of reduced iron stores, blood donors are potentially at risk of developing iron deficiency anaemia. Consequently, this study aims at evaluating the pre- and post-donation ferritin and packed cell volume of blood donors attending University of Calabar Teaching Hospital Donor Clinic.

Method

Study design and setting: The present study made use of descriptive longitudinal design with multistage sampling technique. First, we purposively chose blood donors attending Donor clinic UCTH, then we sampled systematic random sampling to recruit every 3rd donor who visited and gave consent. The study was conducted at University of Calabar Teaching Hospital Donor Clinic from April to June, 2021. University of Calabar Teaching Hospital is a 410 – bed space tertiary health institution located in Calabar metropolis, Calabar, Cross River State, Nigeria.[18,19] Cross River State is in Southern Nigeria.

Study Population: A total of eighteen (18) blood donors attending UCTH Donor Clinic were enrolled for this study. This includes male and female donors aged between 18 to 45 years of age. The subjects were followed up viz: 6 hours post-donation, 7 days post-donation, 14 days post-donation and 30 days post-donation.

Data Collection: A well-structured questionnaire was used to retrieve subject demographic and blood donation history data.

Sample Collection: Approximately 7 ml of blood was collected via venipuncture at 6 hours post-donation, 7 days post-donation, 14 days post-donation and 30 days post-donation. Approximately 3 ml was transferred to EDTA container for packed cell volume (PCV), while the remainder was transferred to plain container for serum extraction. Serum was extracted by centrifugation after clotting of blood. Resultant sera were transferred into plain vials and stored at -20°C for ferritin analysis.

Definition of Donor Type: Voluntary non-renumerated blood donors refer to individuals who donated blood or blood component(s) out of freewill without exchange in the form of payment of cash or kind which could be considered a substitute for money.

Family replacement donors refers to either friend, or relatives of the patient who donate blood or blood

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component(s) as requirement of their patient's transfusion without being paid in cash or in kind.

On the other hand, commercial donors refer to those who donate blood or blood component(s) in return for payment of cash or other benefits that satisfy a basic need.[20] The World Health Organization definition of iron deficiency for adults within 20 – 59 years which has a lower cut-off serum ferritin of <15.0 ng/ml was used.

Determination of Serum Ferritin: Serum ferritin levels were estimated using human ferritin enzyme linked immunosorbent assay kit from Calbiotech Inc (USA) following the manufacturer's instruction. The ferritin kit is based on principle of solid phase enzyme linked immunosorbent assay and utilizes rabbit anti-ferritin for solid phase (microtiter well) immobilization and mouse monoclonal anti-ferritin in the antibody – enzyme (horseradish peroxidase) conjugation solution.

Determination of the packed cell volume: The packed cell volume estimation was performed using the microhematocrit method. This measures the ratio of packed red cell in relation to the entire blood volume in a capillary tube after centrifugation.

Statistical Analysis: We analyzed data generated in this study using SPSS version 25 (SPSS Inc., Chicago, IL, USA). The categorical variables were expressed as frequencies and percentages while continuous variables were expressed as means ± standard deviation. Difference between means across the different days of analysis was performed using repeated measure ANOVA with Greenhouse-Geisser correction. In cases of significant difference, post hoc analysis was performed using Bonferroni adjustment. Logistics regression was used to assess predictors of early recovery of ferritin and packed cell volume to 75% and 100%, respectively, of the baseline values. Trend of values were represented in graph figures. The alpha value was fixed at 0.05.

Result

In total, 18 donors were recruited for the study. However, 5 of the subjects skipped day 14, while 4 were lost on follow up on day 30. Consequently, the computation for day 30 post donation was based on 14 subjects. Among the 18 subjects, 15 were males while 3 were females. Majority of the subjects (55.5%) were within the age range of 26 – 35 years. Most of the donors were repeat donors, mostly within the donation range of 2 – 4 times. Commercial donors constituted most of the donors (50.0%). None of the donors were on iron supplement (Table 1).

We observed a progressive decline in ferritin post donation. The decline continued until day 14 when recovery was noticeable (Figure 2). A repeated measure ANOVA with Greenhouse-Geisser correction determined that the mean ferritin concentration differed significantly between the different days of analysis ($F(2.101, 21.010) = 4.487$, $P = 0.022$). Post hoc analysis with Bonferroni adjustment revealed that ferritin significantly decreased from pre-donation to day 7 (34.34 (95% CI, 3.82 – 64.85) ng/ml, $P = 0.024$) and day 13 (52.76 (95.0% CI, 5.09 – 100.44) ng/ml, $P = 0.027$), but not from pre- donation to 6 hours after donation (41.40 (95% CI, -18.19-100.99) ng/ml $P = 0.321$) and Day 30 (36.90 (95% CI, -8.53-82.33) ng/ml, $P = 0.165$) post donation (Table 1).

There was a significant decline in packed cell volume post donation. The decline was from the 6 hours post donation through day 7. Recovery of packed cell volume was noticeable after day 7 post donation (Figure 3). A repeated measure ANOVA with Greenhouse-Geisser correction showed that the mean packed cell volume differed significantly between the different days pre – and post donation ($F(1.95, 19.52) = 13.750$, $P < 0.01$). Post hoc analysis with Bonferroni adjustment showed that PCV significantly decreased from pre – donation to 6 hours post-donation (1.67 (95% CI, 0.19 – 3.16) %, $p = 0.024$) to 7th day (5.41(95% CI, 2.2 – 8.60) %, $p = 0.001$) and day 14 post-donation (3.86 (95% CI, 0.28 – 7.45) %, $p = 0.032$), but not Day 30 post donation (1.11(95% CI, -0.71 – 2.93%, $p = 0.538$). The pre – donation and 30 days post – donation PCV were comparable (Table 1).

Analysis of iron deficiency among the studied subjects who have been deemed fit to donate by PCV judgement showed 5.6% (n = 1/18) had iron deficiency. However, post donation analysis showed 11.1 % (n = 2) and finally back to single donor deficiency in iron (Table 3).

Further probe showed that none of the donors returned to 100% of their baseline ferritin value by the 30th day analysis. We found that only 43.75%, 75.0% and 100% of the subjects had returned to 75%, 50% and 25% of their baseline ferritin values. On the other hand, 25% and 100% had returned to their 100% and 25 – 75% of their baseline PCV by 30th day evaluation (Table 4).

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On probing for possible prediction of early return to baseline ferritin, none of the demographic variables predicted early return to baseline ferritin within 30 days (Table 5).

Logistics regression on the prediction of early return to baseline PCV showed none of the demographics parameters to be predicted of early return of baseline PCV within 30 days (Table 6).

Table 1: Demographic characteristics of the studied subjects

Variables	Frequency (%)
Age	
18-25	5 (27.8)
26-35	10 (55.5)
36-45	3 (16.7)
Sex	
Male	15 (83.3)
Female	3 (16.7)
Marital Status	
Single	14 (77.8)
Married	4 (22.2)
Status of Iron Supplementation	
Yes	0 (0.0)
No	18 (100.0)
Frequency of Donation	
First time	5 (27.8)
2-4 times	9 (50.0)
>4 times	4 (22.2)
Type of Donor	
Voluntary	7 (38.9)
Commercial	9 (50.0)
Family replacement	2 (11.1)

Table 2: Comparison of ferritin and packed cell volume of the studied subjects within the study time interval.

Variable	Pre-donation n=18	Post-donation				P-value
		6 hours PD n=18	Day 7 PD n=18	Day 14 PD n=13	Day 30 PD n=14	
Ferritin (ng/ml)	115.7 ± 78.5[a]	83.6 ± 47.9[a,b,c]	74.1 ± 67.4[b,c]	55.4 ± 53.2[b]	76.1 ± 56.9[a,c]	0.022
PCV (%)	44.4 ± 3.8[a]	42.9 ± 3.1[b]	39.5 ± 3.2[c]	40.6 ± 4.2[c,d]	44.4 ± 2.6[a,d]	<0.01

PD: *Post donation*

Table 3: Analysis of iron deficiency anaemia using serum ferritin cut off of <14 ng/ml

Anaemia status	Pre-donation n=18	Post-donation			
		6 hours PD n=18	Day 7 PD n=18	Day 14 PD n=13	Day 30 PD n=14
Anaemic)	1 (5.6)	2 (11.1)	2 (11.1)	2 (15.4)	1 (7.1)
Non-anaemic	17 (94.4)	16 (88.9)	16 (88.9)	11 (84.6)	13 (92.9)

PD: Post donation

Table 4: Proportion of the subjects that have returned to 100%, 75%, 50% and 25% of their baseline ferritin and PCV

Parameter	Proportion of baseline returned at day 30			
	100%	75%	50%	25%
Ferritin	0 (0.0)	7 (43.7)	12 (75.0)	16 (100.0)
PCV	4 (25.0)	16 (100.0)	16 (100.0)	16 (100.0)

Table 5: Predicting factors to 75% of ferritin within 30 days of study

Variable	Total no.	No. returned	Not returned	OR	P-value	CI
Sex						
Male	13	3 (38.5)	8 (61.5)	0.313	0.389	0.220-4.413
Female	3	2 (66.7)	1 (33.3)	1		
Age						
18-25	5	1 (20.00)	4 (80.0)	0.125	0.420	0.005-3.225
26-35	8	4 (50.0)	4 (50.0)	0.550		0.031-7.994
36-45	3	2 (66.7)	1 (33.3)	1		
Frequency of Donation						

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Variable	Total no.	No. returned	Not returned	OR	P-value	CI
First time	5	2 (40.0)	3 (60.0)	0.333	0.688	0.017-6.657
2-4 times	8	3 (37.5)	5 (62.5)	0.300		0.018-4.908
>4 times	3	2 (66.7)	1 (33.3)	1		
Type of Donor						
Voluntary	7	1 (14.3)	6 (85.7)	0.000	0.291	0.000-0.000
Commercial	7	4 (57.1)	3 (42.9)	0.000		0.000-0.000
Family replacement	2	2 (100.0)	0 (0.0)	1		

No.: Number; CI: Confidence interval; OR: Odds ratio

Table 6: Predicting factors to 100% of ferritin within 30 days of study

Variable	Total no.	No. returned	Not returned	OR	P-value	CI
Sex						
Male	13	3 (23.1)	10 (76.9)	0.600	0.713	0.390-9.156
Female	3	1 (33.3)	2 (66.7)	1		
Age						
18-25	5	1 (20.00)	4 (80.0)	0.500	0.916	0.019-12.898
26-35	8	2 (25.0)	6 (50.0)	0.667		0.037-11.936
36-45	3	1 (33.3)	2 (33.3)	1		
Frequency of Donation						
First time	5	1 (20.0)	4 (80.0)	0.000	0.807	0.000-0.000
2-4 times	8	3 (37.5)	5 (62.5)	0.000		0.000-0.000
>4 times	3	0 (0.0)	3 (100.0)	1		
Type of Donor						
Voluntary	7	2 (28.6)	5 (71.4)	0.000	1.000	0.000-0.000
Commercial	7	2 (28.6)	5 (71.4)	0.000		0.000-0.000
Family replacement	2	0 (0.0)	2 (100.0)	1		

No.: Number; CI: Confidence interval; OR: Odds ratio

Discussion

In the present study, we found that serum ferritin progressively decreases post donation until about 14 days before recovery. More so, we found that none of the subjects returned to the baseline ferritin at 30th day of assessment. This finding buttresses further the need to widen inter -donation interval to allow full recovery of ferritin following whole blood donation. Some European and North American countries have a standing guideline of ≥ 56 days inter-donation interval.[22,23] However, a study by Scholten and Colleagues as well as the report of the Recipient Epidemiology and Donor Evaluation Study – III (REDS - III) highlights the unsuitability of donation intervals less than 180 days [24]. Similar to the present study, Scholten and colleagues reported that 25 – 32% of the studied subject returned to baseline ferritin at day 57 while 100% returned to baseline at 180 days.[24]

We found one of the blood donors (commercial donor) who was found fit by packed cell volume assessment to be iron deficient. This further highlights the unsuitability of Hb and PVC (hematocrit) as a measure of iron reserve. More so, while none of the donors returned to baseline ferritin at day 30, approximately 25% of the donors have returned to their baseline PCV.

This disparity between recoveries of ferritin can be explained by the existing theory of recovery of blood losses. During blood loss, (mostly acute), there is fast decrease in haemoglobin Hb / PCV owing to dilution to replace loss in blood volume, followed by hypoxia induced erythropoietin production and increase in erythropoietin – induced erythropoiesis and associated increase in erythrocytes, hepcidin decreases via both signaling of increased erythropoiesis to hepatocytes and decrease in body iron levels and subsequently, ferritin decreases via low – hepcidin induced release of stored iron into plasma. All these leads to increased erythropoiesis and iron availability for uptake in newly synthesized erythrocytes, leading to return in the Hb / PCV earlier and subsequently with time other iron parameter including ferritin to pre – donation value.[24,25] Aside blood donation, there have been other

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documented factors influencing serum ferritin are ethanol intake in men and diet in women.[26]

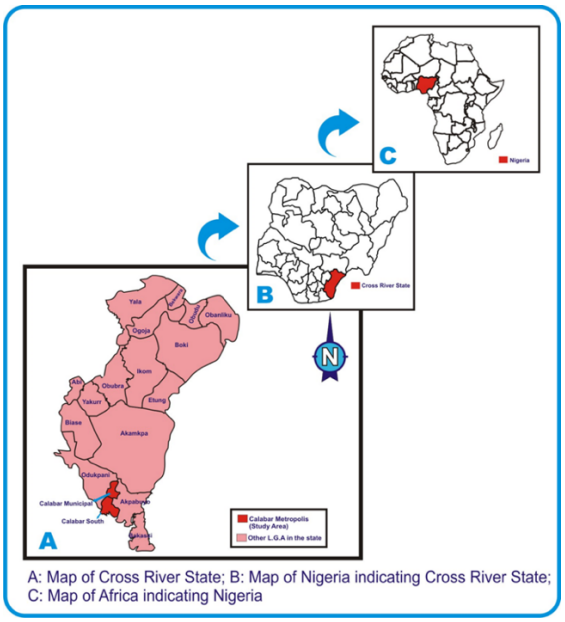

Figure 1: Map of the studied area

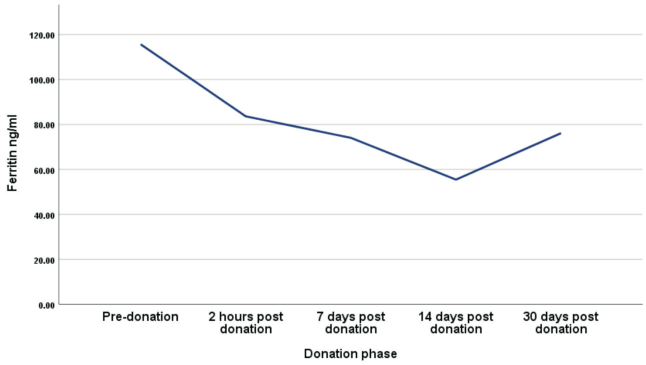

Figure 2: Trend of ferritin along pre and post donation periods

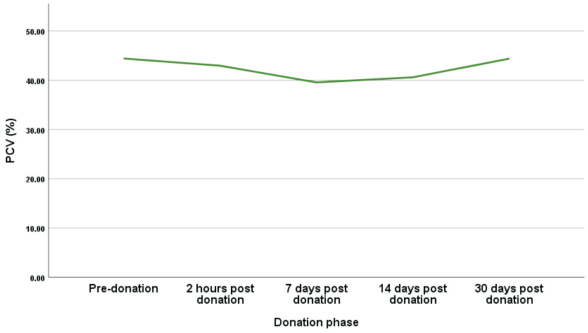

Figure 3: Trend of packed cell volume (PCV) along pre and post donation period.

Limitations of study: The findings of this study need to be interpreted in view of a limitation. We did not perform reticulocytes count. This would have given an insight on the bone marrow response during the physiological recovery stages. More so, a study with larger sample size may aid in confirming the findings of this study.

Conclusion

This study has shown that there is significant negative change in serum ferritin level of blood donors after donation and same doesn't recover fully within 30 days post donation. More so, we find that packed cell volume (as often used in Nigeria) is not suitable to access donor's safety prior to donation. We therefore recommend serum ferritin as part of screening process and iron supplementation following blood donation, especially those who intend to repeat donation.

Declarations

Ethical consideration: This study was approved by the Health Research Ethics Committee of University of Calabar Teaching Hospital.

Authors' contribution: Conceptualization: OIM, AVO; Sample analysis: OIM, AVO, OHU, OCO; Supervision: OIM; Data analysis: OHU, OIM; Literature search: AVO, OHU, OIM; Manuscript draft: OIM, OHU. Resources: AVO, OIM; All authors read, edited and approved the final manuscript before submission.

Conflict of interest: The authors declare that they have no competing interests.

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Availability of data and materials: Data sets generated in this study are available from the corresponding author on request.

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