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A Study of Serum Ferritin Levels in Blood Donors in Orlu, Imo State

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Abstract
Haemoglobin levels, PCV and serum ferritin levels were measured in three groups of individuals. These groups are: (a) individuals with no history of donation (Group A), (b) individuals with a history of 1-3 donations (Group B) and individuals with a history of 4- 7 donations in the last one year (Group C). The mean haemoglobin levels in group A, group B, group C is 14.05±1.22g/dl, 14.03+ 1.00g/dl and 11.75±1.30g/dl respectively. The mean PCV level in group A, B and C were 41.80±3.83%, 41.60±3.61% and 34.40±5.04% respectively while the mean serum ferritin levels of group A are 84.14±45.10 ng/ml, group B 68.35±35.06 ng/ml and group C 7.79±9.24 ng/ml. The WHO reference range for each parameter was obtained as HB (males) = 13.0 – 18.0g/dl, PCV (males) = 40 – 54%, serum ferritin (males) = 21-38.5 ng/ml. The serum ferritin level decreased according to the number of donations. The serum ferritin levels were found to be significantly decreased ($P<0.05$) among the group C when compared to group A. There was also a significant decrease ($P<0.05$) in haemoglobin level and PCV of the group C (11.75±75 g/dl and 34.40±5.04% respectively) when compared to group A (14.05±1.22g/dl and 41.80±3.83%). Sixty percent of donors in group C had their iron stores (serum ferritin <4 ng/ml) completely depleted and had developed iron deficiency anaemia (HB<13.5g/dl). However, from this study it was observed that iron deficiency was high in donors donating above the stipulated 2-3 donation per year.

Keywords: *serum iron, ferritin, blood donors*

Introduction
Iron is the most abundant of all essential elements in animal tissues and participates in many biochemical processes including redox reactions. It is a universal cofactor for mitochondrial energy generation and support the growth and differentiation of all cell types. Iron is an essential component in the synthesis of haemoglobin, myoglobin, and several haem and metalloflavoproteins and its turnover in the human body is dominated by the synthesis and breakdown of haemoglobin. Every day about 30mg of iron is used to make new haemoglobin, and most of this is obtained from the breakdown of old red blood cells.[1] Iron enters the body after absorption from the diet and it is highly conserved in humans. The total body iron content ranges between 2 and 4 g: approximately 50 mg/kg in men and 35 mg/kg in women. The iron content of the body is distributed among various proteins in the body. Approximately 100-1000 mg of iron

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is stored in form of ferritin and haemosiderin in the liver, spleen, and bone marrow. About 200-400 mg of iron is present in myoglobin and non haem enzymes.[2] Most (70-90%) of iron is present in haemoglobin in circulating red blood cells. According to Mcphee *et al*3, one milliliter of packed red blood cells contains approximately 1 mg of iron. A 70-kg man will therefore have approximately 2100ml packed red cells and consequently 2100mg of iron in his circulating blood.

A donor generally donates approximately 450ml of blood at the time of donation. One gram of haemoglobin contains 3.4mg of iron.[1] In a normal individual with 15g of haemoglobin per dl, 100 ml of blood contains approximately 50 mg of iron. Thus, removal of only 2 ml of blood results in the loss of 1 mg of iron.[4] If 450 ml of blood is taken in a donation, approximately 225 mg of iron will be lost. If the donor has no iron deficiency, the erythrocytes and the haemoglobin level will generally return to normal within 3-4 weeks. The physiological importance of the storage iron is that it provides a rapidly available supply of iron in the event of blood loss.[5] Serum ferritin is an indicator of mobilizable body iron stores. Acceptable frequency of donation is normally two or three times a year.[6] This enables a donor replenish his iron store and prevent the development of iron deficiency anaemia. Hence, adequate iron stores are very important in maintenance of donors' health.

Materials and Method

Study Area

The study was conducted at Imo State University Teaching Hospital Umuna in Orlu Local Government Area.

Study Population and Recruitment

A total of 60 populations (all males) aged 18-40 years were used for this study. These populations were divided into three groups of study (group A, group B and group C). The first group consists of 20 individuals with no history of blood donation (non-blood donors) whom were used as the control group. The second group consists of 20 voluntary blood donors who have donated blood 1-3 times in the last one year. The third group consists of 20 commercial blood donors, who have donated blood 4-7 times in the last one year. The samples for study (commercial and voluntary blood donors) were collected from blood donors in Blood Transfusion unit of Imo State Teaching Hospital (IMSUTH), Orlu, while the non-blood donor's samples were collected from individuals in Orlu. Their medical histories were noted and those who were healthy were used for the study.

Inclusion Criteria

Individuals who have donated blood in the last one year with normal or low PCV and Hb levels.

Exclusion Criteria

Pregnant women, menstruating women, individuals who are medically ill, people below or above the age limit of 18 – 40 years respectively and individuals who refuse to give their consent were all excluded in the study.

Sample Collection and Preparation
Using a 10ml sterile syringe, 5ml of fresh venous blood was drawn from each subject by a clean vein puncture from the antecubital vein and delivered into two different containers; one with EDTA and the other in an iron free dried plain tube. The EDTA specimens were used for the estimation of haemoglobin, and packed cell volume (PCV). The sample in plain test tubes were allowed to clot at room temperature and centrifuged to separate the serum. The serum was transferred into clean test tubes for the determination of serum ferritin. The samples were then taken to Medical Laboratory Science Laboratory, Imo State University (IMSU) Owerri where the tests were carried out.

Laboratory methods and procedures
The reagents for the research were commercially purchased and manufacturer's standard operating procedures were strictly followed.

Serum Ferritin Estimation: Reagent kit was purchased from BIOTEC Laboratories Ltd. Catalog No 7/352.

Haemoglobin Estimation
The cyanmethaemoglobin method was used for the haemoglobin estimation.

Procedure
1 in 201 dilution of blood were made by adding 20 µl of blood to 4 ml of diluent (Drapkin's solution) into a test tube. The test tubes were stoppered and inverted several times and were then poured into a cuvette and the absorbance were read in a colorimeter at 540 nm against the reagent blank. Haemoglobin level was read off from the calibration graph prepared.

Haematocrit or Packed Cell Volume (PCV) Measurement
The micro-haematocrit method was used for the estimation of PCV.

Procedure
About three quarter of a plane capillary tube were filled with a well-mixed EDTA anti coagulated blood. The empty ends of the tubes were sealed with modellin clay (plastercin). The tubes were placed in a special groove provided in the micro-haematocrit centrifuge and centrifuge for 3-5 minutes at 10, 000rpm. After centrifuging, the PCV were read off using the micro-haematocrit reader.

Serum Ferritin Estimation
The serum ferritin was estimated by Enzyme-Immunoassay method.
Procedure
Into different microplates, 20µl of the test samples and standard solution were added. An anti-ferritin monoclonal antibody conjugate of 100 µl was added into each standard and test microplate. The microplates were sealed using plate sealer and were shaken for 30 seconds to allow proper mixing of the solution. The mixtures were in an incubator at 37^0C for 30 minutes. Using microplate washer, the test and standard plates were washed with washing solution to remove unbounded

antibodies. 200 µl chromogen / substrate solution was added into each microplate and sealed with plate sealer. The solution was incubated for 10 minutes at room temperature in the dark. 100 µl of stop solution was added after 10 minutes to terminate the enzyme reaction and a coloured solution was obtained. The concentration of ferritin in each sample was measured using automatic microplate reader. The colour intensity is proportional to the concentration of the ferritin present in each sample.

Statistical analysis

The values were expressed as mean standard deviation and the test of significance was carried out using the student T- test.

Results

Table 1: Classification of studied subjects relative to the number of donations in the last one year

Group	Category	Number of Donation in the last one year	Total Number Tested
A	no history of donation	0	20
B	Voluntary donors	1-3	20
C	Commercial donors	4-7	20

Table 1 shows the classification of the individuals tested. From a total of 60 samples, 20 were from non-donors of normal subjects (control group), 20 samples came from voluntary blood donors, and 20 from commercial blood donors. It was further grouped into A, B and C based on the number of donation in the last one year.

Table 2: Mean and standard deviation of serum ferritin, Hb, and PCV of the non-donors (control), voluntary and commercial donors

Group	Number of Donation in the last one year	Serum Ferritin	Hb	PCV
A	0	84.14 ±45.10	14.05 ±1.22	41.80 ±3.83
B	1-3	68.35 ±34.06	14.03 ±1.00	41.60 ±3.61
C	4-7	7.79 ±9.24	11.75 ±1.30	34.40 ±5.04

Table 2 shows the mean ±S.D values for serum ferritin level, haemoglobin (Hb) concentration and PCV tested for in group A, B, and C. In group A, the mean ±S.D values of serum ferritin, Hb and PCV were 84.14 ±45.10, 14.05 ±1.22, and 41.80 ±3.83 respectively. In group B, the mean ±S.D of serum ferritin, Hb and PCV were 68.35 ±34.06, 14.03 ±1.00, and 41.60 ±3.61 respectively. The mean ±S.D of serum ferritin, Hb and PCV of group C were 7.79 ±9.24, 11.75 ±1.30, and 34.40 ±5.04 respectively.

Table 3: Comparison of serum ferritin, Hb and PCV of Group A with Group B

	Serum ferritin	Hb	PCV
T-cal	-1.250	-0.06	-0.17
T-tab	±1.960	±1.960	±1.960
P-value level	P>0.05	P>0.05	P>0.05
Significant	__	__	__

Key:
T-cal = T-Calculated
T-tab = T-Tabulated
P<0.05 (+) = Significant
P>0.05 (-) = Not Significant

From table 3, there was no significant (P>0.05) decrease in serum ferritin level of the group B (68.35 ±34.06) when compared to control group (84.14 ±45.10). There was no significant (P>0.05) decrease in haemoglobin (Hb) level of the group B (14.03 ±1.00) when compared to group A (14.05 ±1.22). Also there was no significant (P>0.05) decrease in the PCV of the group B (41.60 ±3.61) when compared to group A (41.80 ±3.83).

Table 4: Comparison of serum ferritin level, Hb level and PCV of group A with group C

	Serum ferritin	Hb	PCV
T-cal	-7.42	-5.75	-5.21
T-tab	±1.960	±1.960	±1.960
P-value level	P<0.05	P<0.05	P<0.05
Significant	+	+	+

Key:
T-cal = T-Calculated
T-tab = T-Tabulated
P<0.05 (+) = Significant
P>0.05 (-) = Not Significant

From table 4, there was a significant (P<0.05) decrease in serum ferritin level of the group C (7.79 ±9.24) when compared to control group (84.14 ±45.10). There was a significant (P<0.05) decrease in haemoglobin (Hb) level of the group C (11.75 ±1.30) when compared to group A (14.05 ±1.22). Also there was a significant (P<0.05) decrease in the PCV of the group C (34.40 ±5.04) when compared to group A (41.80 ±3.83).

Discussion

From the result obtained, the mean serum ferritin levels decreased in the group C blood donors (7.79 ±9.24) who have made up to 4 to 7 donations in the last one year, as compared to the mean serum ferritin level in group A (84.14 ±45.10) and group B (68.35 ±34.06). The value of serum ferritin obtained from the voluntary donors (group B) shows that at normal donation interval (2-3 times per year) the iron stores remained within normal range. However, the result from this study indicated that there was a significant relationship between the frequency of donation and the serum ferritin level. After a single donation, a person needs approximately 3 months to replenish iron stores. With continued bleeding below the 3 months interval, an individual either reaches equilibrium at a lower level of iron stores or becomes anaemic. It is not surprising therefore; that the value of serum ferritin obtained from group C in this study was far less than what was obtained from their counterpart in Group B. The values could not even be compared with those of Group A. It is important to note that the lowest serum ferritin level came from Group C. The values obtained from this study give a confirmatory high loss of body's iron stores through regular blood donation. Several studies had been carried out confirming iron stores depletion in blood donors.[7-

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[9] The previous studies by Milman *et al*[10], and Alvarez-Osorio *et al*[9], show that the serum ferritin was reduced markedly in regular donors corresponding to annual donation frequency.

The cut-off value for blood donation in men for haemoglobin level and PCV are 13.5 g/dl and 40-54% respectively. The mean haemoglobin level and PCV of group A (Hb=14.05±1.22, PCV= 41.80±3.83) when compared to the mean haemoglobin level and PCV of group C (Hb=11.75±1.30, PCV=34.40±5.04) showed that there was a significant decrease (P<0.05) in haemoglobin level and PCV with number of donations. This confirms the previous studies that there is a significant relationship between the number of donations and haemoglobin level. As shown by the result obtained, there was no significant relationship between haemoglobin level, PCV and serum ferritin concentration unless in severe iron deficiency (serum ferritin < 4 ng/ml). The results also show that at a very low serum ferritin below the normal range (<15.2 ng/ml) in group C, haemoglobin level and PCV were normal (13.5 g/dl and 43 %) (see raw data). These individuals had passed the haemoglobin and PCV screening prior to blood donation, but serum ferritin levels were found to be significantly low in these donors (serum ferritin 7.79 ±9.24). Low haemoglobin level and PCV alongside low serum ferritin was observed in 60 % of the commercial blood donors. Their iron stores are completely depleted (serum ferritin<4 ng/ml). These individuals have developed iron-deficiency anaemia (Hb<13.5) and were not deemed eligible for blood donation hence, were not bled. Several studies indicate that haemoglobin is not a sensitive indicator to detect iron deficiency at an early stage, but is useful in detecting the majority of blood donors with established iron deficiency.[7,11]

Conclusion

A high prevalence of iron store deficiency is present among commercial blood donors. The serum ferritin levels gradually decrease according to the number of donations and there was a significant relationship between frequency of donations and the serum ferritin level. The association between haemoglobin (Hb) levels, PCV and iron deficiency was poor and the screening test was found insensitive to iron stores depletion with some of the donors passing the test and donating blood despite being iron deficient.

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