

Correlation of Serum Erythropoietin and Ferritin Levels with the Frequency of Blood Donation

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Abstract

Aim: The objective is to assess the correlation of serum ferritin and erythropoietin levels with the frequency of donation among blood donors in Pulau Penang, Malaysia. **Methods:** One hundred and forty-seven (108 male and 39 female) voluntary whole blood donors whom donating blood at the Blood bank, Hospital Pulau Pinang were included. For each donor, haemoglobin, serum ferritin and erythropoietin (EPO) assay were done. Laboratory works had been done at the Advanced Diagnostic Laboratories (ADL), Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM).

Results: A significantly increased ($p=0.014$) serum ferritin was found in the first time donor (105.3 ng/ml) compared to regular donors (69.3 ng/ml) who had donate blood more than once in the preceding year of both gender. The larger amount of donated blood in regular male donor results in a more risk of latent iron deficiency compared to female counterpart. EPO was significantly higher while ferritin was significantly lower in regular donors than in the control. The serum EPO showed a statistically significant inverse relationship with ferritin level and this relationship was significantly correlated.

Conclusion: Adequate daily, oral iron supplementation at post donation would need to be provided for 90 days, which is longer than most current recommended iron replenishment programs together with the introduction of serum ferritin assay at first donation and annually for regular donors to increase the safety of national blood supply recommended.

Keywords: Erythropoietin; Ferritin; First time blood donor; Regular blood donor; Malaysia

Introduction

Recruiting more regular blood donors and encourage first-time donors to become repeat donors is the steady push for increasing the quantity and safety of national blood supplies. Iron deficiency is a well-recognized risk factor of repeat blood donation. Whole blood donation of 450 ml results in a loss of about 230 mg iron from the total-body iron [1]. The donation of 200 ml of red blood cells four times a year is equivalent to an iron loss of 2.2 mg of iron per day. This is substantially more than the normal daily absorbed iron for men (1 mg) and women (1.5 mg) [2]. The increase in the frequency of blood donations among donor population is liable to result in excessive iron loss and development of latent iron deficiency as well as iron deficiency anaemia [3]. Measurement of donor haemoglobin (Hb) is required to ensure that the recipient will receive an adequate red cell mass, to detect donor abnormalities in red cell or haemoglobin, and it serves as a non-specific measure of donor health. Furthermore, it ensures that the expected drop in haemoglobin will not be harmful to the donor, and it is an indirect measure of iron stores [4]. However, haemoglobin estimation alone may defectively evaluate donor body iron status prior to phlebotomy with potential risk for developing latent iron deficiency: a state in which iron stores are depleted and plasma iron is decreased but haemoglobin remains normal [5]. Szymczyk-Nuzka and Wolowicz [6] studied the iron balance in regular blood donors with normal haemoglobin and hematocrit levels who had given over 10 donations of the whole blood with frequency of 4-6 units per year and compared with control group first time donor. The following differences were found: total iron binding capacity, serum transferrin, soluble transferrin receptor, erythropoietin were significantly higher while the mean corpuscular volume, serum ferritin and transferrin saturation

were significantly lower in regular donors than in control. The erythropoietin (EPO) mediates the remarkable regenerative capacity of the bone marrow to compensate for blood loss or reduced oxygen tension [7]. The erythropoietin level does not increase detectably after phlebotomy of a subject with no erythropoietic defect of 500 ml whole blood and the red cell mass will slowly recover after losing 500 ml blood. Accordingly, 2.0 ml of the lost 240 ml RBCs will be recovered daily [8]. The EPO level is inversely correlated with haemoglobin concentration [9]. It would take 4 years or more for a man to deplete body iron stores and start developing to iron deficiency anaemia solely due to lack of dietary intake or malabsorption [10]. Initially, less than optimal level of iron leads to a decrease in iron stores and progresses to the latent iron deficiency [11]. The amount of circulating ferritin parallels the concentration of storage iron in the body. Therefore, serum ferritin concentration is a reliable index of iron stores (1 ng/ml of serum ferritin indicates about 8 mg of storage iron) [12,13]. Serum ferritin does not exhibit diurnal variation as are seen with serum iron levels [14]. Low serum ferritin is highly specific for body iron status assessment [15,16]. Flesland 2004 measures the haemoglobin, serum

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ferritin and transferrin receptor (sTfR) immediately at post-donation of six or more units of whole blood during the previous three years. They found that 51% of repeat male donors and 11% of female repeat donors had low tissue iron stores, but only 7% and 5% had functional iron deficiency indicated by elevated sTfR [4]. The majority of these had normal Hb values. Furthermore, they reported that the female repeat donors suffer less iron loss than male repeat donors. Mittal et al. [3] reported that deficient iron store was found more frequently in female donors compared to male donors. The serum ferritin levels are higher in men than in women of childbearing age, reflecting differences in storage iron. Levels in postmenopausal women are closer to those found in men [14]. The aim of this study was to assess the correlation of serum ferritin and erythropoietin levels with the frequency of donation among blood donors in Pulau Penang, Malaysia.

Methodology

Blood samples from blood donors were taken after ethical approval by Human Research Ethical Committee, Universiti Sains Malaysia (USM), Malaysia. Laboratory works had been done at the Advanced Diagnostic Laboratories (ADL), Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM). One hundred and forty-seven (108 male and 39 female) voluntary whole blood donors (74 regular donors and 73 first-time donors) donating blood at the Blood bank, Hospital Pulau Pinang were recruited in this study. Sample size was calculated using power and sample size program (PS version 3.0.43) [17]. Donors Inclusion criteria are: Haemoglobin between 12.5 g/dl-8.0 g/dl and the interval of the last donations of whole blood should not be less than 8 weeks [13]. Regular donors defined as those who have at least two previous donations within the past year and first-time donors are those who had never made a donation before. Hemoglobin concentration results were collected from the blood bank records for each donor. Hemoglobin level was determined using Hemocue Hb 301 analyzer and HemoCue Hb 301 microcuvettes. The serum erythropoietin level among the first time and regular donors (i.e. donates blood more than once per year) in this study was measured using (Human Erythropoietin Quantikine IVD ELISA Kit, DEP00, USA). A three ml venous blood was collected in gel separator plain tubes from the diversion pouch at the beginning of the donation. After serum preparation within less than three hours, the serum samples were kept frozen at -20°C till the time of analysis within three weeks after the collection date. The serum ferritin assay done using Abbott Architect immunoassay analyzer i2000 SR (ARCHITECT Ferritin Kit (7K59)) using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Statistical Analysis was conducted using SPSS version 18. A p-value of less than 0.05 was considered statistically significant.

Results

All Obtained results were summarized in the group as below

Discussion

The significantly higher ($p=0.014$) levels of serum ferritin in the first time donor (105.3 ng/ml) compared to the regular donors (69.3 ng/ml) who had donate blood more than once in the preceding year of both gender (Table 1) is supported by the additional finding of a statistically significant inversely relationship of serum ferritin and proportionally relationship of erythropoietin levels with the frequency of donation per year (Table 2). The serum ferritin levels were significantly higher in female who had donated more than 3 times over a one year period compared to male donors (Figure 1). This finding

might attributes to the larger volumes of blood that males donate in the transfusion unit as compared to females. Males generally donate 450 ml of blood as compared to females who donate 250-300 ml of blood unless their body weight exceeds 55 kg [18]. Iron depletion is the earliest stage of iron deficiency. Latent iron deficiency without anaemia is a somewhat more advanced stage of iron deficiency and indicates that iron stores are decreased or absent, but the serum iron concentration and blood haemoglobin levels are normal [19]. The most frequent observation in long-term blood donors is chronic iron deficiency [20-22]. The haemoglobin cut off levels (12.5 g/dL) does not appear to be positive predictor of iron deficiency as a significant proportion of individuals with low ferritin levels showing haemoglobin levels above 12.5 g/dL (Table 3). This finding might be caused by the inherent limitation of the Hemocue instrument used for donor screening in our blood center. The latter is supported by other studies [23-26] which show that the Hemocue gives higher haemoglobin levels

Table 1: Haemoglobin, serum erythropoietin and ferritin levels in the first-time and regular donors.

Blood donors		No./total (%)	Hb (g/dl) mean \pm SD	Ferritin (ng/ml) mean \pm SD	EPO (mIU/mL) mean \pm SD
First-time	Male	50/73 (68.5)	15.8 \pm 1.0	129.5 \pm 72.1	7.9 \pm 3.9
	Female	23/73 (31.5)	13.6 \pm 0.9	52.5 \pm 34.7	8.7 \pm 4.5
	Total	73/147 (49.7)	15.1 \pm 1.4	105.3 \pm 72.1	8.2 \pm 4.1
Regular	Male	58/74 (78.4)	15.4 \pm 1.2	78.5 \pm 70.2	8.0 \pm 4.4
	Female	16/74 (21.6)	13.6 \pm 0.6	36.1 \pm 33.0	8.4 \pm 6.6
	Total	74/147 (50.3)	15.0 \pm 1.3	69.3 \pm 66.2	8.1 \pm 4.9
P-value			0.055	0.014*	0.481

The mean age of all donors was 31.3 \pm 11.1 (range: 17-59 years). *Statistically significant increased ($p=0.014$) serum ferritin of the first-time compared to regular donors. Hb: Haemoglobin; EPO: Erythropoietin

Table 2: Relationship of haemoglobin, serum erythropoietin and ferritin levels with studied donor groups.

Blood donors		No./total (%)	Hb (g/dl) mean \pm SD	Ferritin (ng/ml) mean \pm SD	EPO (mIU/mL) mean \pm SD	
Group I	Male	50/73 (68.5)	15.8 \pm 1.0	129.5 \pm 72.1	7.9 \pm 3.9	
	Female	23/73 (31.5)	13.6 \pm 0.9	52.5 \pm 34.7	8.7 \pm 4.5	
	Total	73/147 (49.7)	15.1 \pm 1.4	105.3 \pm 72.1	8.2 \pm 4.1	
Group II	Male	10/15 (66.7)	16.0 \pm 1.1	129.1 \pm 95.2	8.0 \pm 5.0	
	Female	5/15 (33.3)	13.3 \pm 0.4	44.3 \pm 39.2	6.4 \pm 1.5	
Group III	Total	15/147 (10.2)	15.1 \pm 1.6	100.9 \pm 89.3	7.4 \pm 4.1	
	Male	20/23 (87)	15.3 \pm 1.3	82.2 \pm 54.5	6.9 \pm 2.5	
	Female	3/23 (13)	13.1 \pm 0.3	11.4 \pm 1.6	15.0 \pm 13.8	
Group IV	Total	23/147 (15.6)	15.0 \pm 1.4	73.0 \pm 56.2	7.9 \pm 5.5	
	Male	15/18 (83.3)	15.3 \pm 1.0	44.3 \pm 23.5	9.2 \pm 5.8	
	Female	3/18 (16.7)	14.3 \pm 0.9	65.4 \pm 42.0	6.5 \pm 1.8	
Group V	Total	18/147 (12.2)	15.2 \pm 1.1	47.8 \pm 27.0	8.7 \pm 5.4	
	Male	13/18 (72.2)	15.4 \pm 1.3	73.3 \pm 87.2	8.3 \pm 4.6	
	Female	5/18 (27.3)	13.7 \pm 0.5	25.0 \pm 18.1	7.8 \pm 4.8	
Total			18/147 (12.2)	14.9 \pm 1.4	59.8 \pm 77.1	8.1 \pm 4.6
p-value			0.133	0.001*	0.001*	

Hb: Haemoglobin, EPO: erythropoietin. *Statistically significant difference of serum ferritin ($p=0.001$) and erythropoietin levels ($p=0.001$) with the frequency of donation per year. Donors were divided into five groups according to the frequency of donation during a one year period preceding this study (April 2011- April 2012): Group I (No.=73) includes the first-time donors. Group II (n=15): includes those who had donated once in the previous year and were donating for the second time with an interval of more than 3 months (Rang: 3 - 6). Group III (n=23) includes those who had donated twice in the previous year and were donating for the third time. Group IV (n=18) involves those who had donated three time in the previous year. Group V (n=18) involves those who had donated four times in the previous year and were donating for the fifth time. Groups II, III, IV and V donors were considered regular donors (n=74)

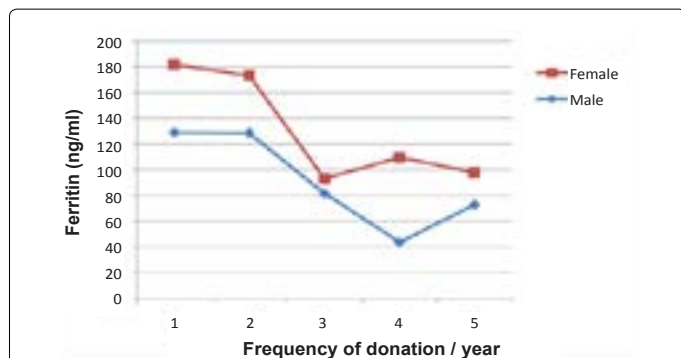


Figure 1: Serum ferritin levels in both genders according to the frequency of blood donation in the studied donors. The serum ferritin is significantly higher in female who had donated more than three times in the preceding year compared to male donors.

Table 3: Correlation of haemoglobin, ferritin levels with erythropoietin levels.

Variable	Gender	No./total (%)	Hb (g/dl) mean \pm SD.	Ferritin (ng/ml) mean \pm SD.	
Serum Erythropoietin	Below-median (low EPO)	Male	84/114 (73.7)	15.8 \pm 1.1	107.6 \pm 78.7
		Female	30/114 (26.3)	13.5 \pm 0.8	46.7 \pm 37.6
		Total	114/147 (77.5)	15.2 \pm 1.4	91.6 \pm 75.0
	Above-median (high EPO)	Male	24/33 (72.7)	14.9 \pm 1.1	83.0 \pm 59.2
		Female	9/33 (27.3)	13.9 \pm 0.7	42.5 \pm 22.9
		Total	33/147 (22.5)	14.6 \pm 1.1	71.9 \pm 54.6
p-value			0.309	0.002*	
Pearson correlation coefficient				-1.81*	

Hb: Haemoglobin, EPO: Erythropoietin. *Statistically significant ($p=0.002$) inverse correlation ($r=-1.81$) of the serum ferritin with the serum EPO levels. Out of the total donors, 114 (77.5%) donors have below-median serum erythropoietin [mean Hb value 15.2g/dl (SD=1.4)] and ferritin mean value 91.6 ng/ml (SD=75) comparing to 33 (22.5%) donors whom have above-median serum erythropoietin [mean Hb value 14.6 g/dl (SD=1.1)] and ferritin mean value 71.9

of the donors compared to the automatic blood analyzer results. In this study, no significant relationship of the age with EPO and Hb levels in the donors was found. The endogenous serum erythropoietin showed a statistically significant inverse relationship with ferritin level ($P=0.002$) which reflects the well-known ineffective type of erythropoiesis that is associated with iron deficiency status [9,11,27-29]. Furthermore, this inverse relationship was with correlation coefficient (r) of -1.81 (Table 3) which suggested that serum erythropoietin assay represent a supportive confirmation test in the evaluation of the body iron status in blood donors. This study found that EPO was significantly higher and the ferritin was significantly lower in regular donors than in the first time donors. These findings agree with that of previous studies [6,8,28,30]. A recently published study reported that daily oral iron supplementation accelerate both hemoglobin and ferritin recovery and it would need to be provided for 90 days, which is longer than most current recommended iron replenishment programs [31]. Parenteral iron may be recommended in case of non-compliance and/or not tolerated daily oral iron intake.

Conclusion and Recommendation

Regular blood donors are more prone to have lower body iron store and development of latent iron deficiency compared to first time donors. The larger amount of donated blood in regular male donor results in a more risk of iron deficiency state compared to female counterpart. Adequate daily oral iron supplementation would need to be provided

for 90 days, which is longer than most current recommended iron replenishment programs. Introduction of serum ferritin assay at first donation and every year for regular donors to increase the safety of national blood supplies by preventing the progression of the regular donors from latent iron deficiency state to iron deficiency anaemia recommended.

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