Influence of Blood Donation on Iron Stores of Female Blood Donors in Enugu

Ngwu Amauche Martina¹

¹Hematology and Immunology Department, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.
muchyscki@gmail.com.

Abstract – Women cannot donate blood as frequently as men. Women in low-income countries in which Nigeria is one of them can donate one to two times per year. The present study was designed to evaluate the iron status in voluntary female blood donors that donated once or twice in one year. This study was done between April 2012 and December 2012 at Hematology and Immunology Unity of Enugu State University of Science and Technology, Enugu (ESUT). Blood samples of 41 voluntary female blood donors (W2 and W3) and 19 apparently healthy female (W1) with no previous history of blood donation (aged 19-29 years) were collected. Data were evaluated with SPSS (version 17) software and comparisons between groups were made by one-way ANOVA. The result of hemoglobin concentration was significantly lower in female blood donors that donated twice in a year when compared with non-female blood donors. Iron deficiency was seen in 3.4% of first-time donor and 8.3% in donors that donated twice in one year. This study reveals the presence of iron deficiency in first and second time’s female blood donors.

Key Words – Female blood donors; Iron deficiency; Ferritin.

1 Introduction

Alan Mast noted that males store on average 1 gram of iron while females store on average 270 mg and iron lost in one donation is approximately 250 mg [1]. Because of lower average storage of iron in women, women can no longer donate blood as frequently as men especially in low-income countries. The result of work done by Marco Bani and Barbara Giussani, the demographic data of their blood donors showed that female blood donors are very few in number compared to males [2]. Reasons like low donor turnover and temporary deferral conditions such as low hemoglobin values, fear of pain and low weight may be responsible for less percentage of female blood donors. Study done by Hollingsworth revealed that female blood donors constituted only 1% of the donor population [3]. The predominant reasons for small number of female to be involved in blood donation was fear of some aspects of the collection process, such as needles, mistakes and feeling unwell, in addition to above reasons others are poverty and illiteracy in developing countries. Those reasons are more prevalent among women. Less than 10% blood donations are given by female in 18 out of 104 countries [4,5]. In low-income countries, the donor population was mainly young people and undergraduate student who were between the ages of 18-25 years [6]. Studies show that women account for about 30.2% of active donors in Trentino, 33% and 67% of women in Veneto and Tuscany, in Spain 46% of the donors are women, in Denmark 50% and in Finland 55%. In African their case is entirely different, about 7% of women accounted for blood donation [6]. In addition to smaller number of women participation in
blood donation in Africa, whom Nigeria is one of them, there is also high prevalence of iron deficiency and iron deficiency anemia in first time and regular female African blood donors [7]. There are non accurate statistics of limiting factors for female blood donations in Enugu, Nigeria and it is not possible to use the result of other studies in other countries for female blood donors in Enugu. Therefore, this study was carried out to evaluate the iron status in first and second time’s female blood donors in Enugu.

2 Subject and methods

Forty one voluntary female blood donors aged 19-29 years that donated blood during the National blood transfusion centre blood drive in Enugu, between April and December 2012 were recruited into the study after given informed consent. Before carrying out the research ethical clearance approval was given by the Enugu State University of Science and Technology Teaching Hospital Ethics Committee. Nineteen apparently healthy unmarried, undergraduate female students with no previous history of blood transfusion served as control (W1). The voluntary female blood donors are made up of unmarried, undergraduate female students schooling in Enugu. The blood donors were 41 and divided into two groups according to the number of donation one had made in one year; group W2 (n=29) had donated one unit of blood in one year, group W3 had donated two units of blood in one year. Data were collected by completion of Questionnaire. In this study, iron depletion, iron deficiency, and iron deficiency anemia are defined below: Iron depletion: Serum ferritin less than 20 ng/mL. Iron Deficiency: Serum ferritin less than 12 ng/mL and transferrin saturation percentage less than 15. Iron deficiency anemia: Serum ferritin less than 12ng/mL, transferrin saturation percentage less than 15, and Hb less than 12.0 g/dL.

- Study population: Enugu State is in the South-East geographical zone of Nigeria. The Enugu metropolitan area has an estimated population of 722,664, according to the 2006 Nigerian census.
- Methods: Serum ferritin measurement was done by human ferritin enzyme immunoassay kit (BIOCHECK, INC, 323 Vintage Park Dr. Foster City, CA 94404). The ferritin quantitative test is based on a solid phase enzyme–linked immunosorbent assay (ELISA). Serum iron and total iron binding capacity (TIBC) were estimated using a ferrozine–based iron/TIBC reagent set (TECO DIAGNOSTICS, USA). Test procedures were conducted as described in the manufacturer's standard operating manual included with the kit.
- Serum Iron Concentration (Spectrophotometric Method)

Principle: The iron in serum is dissociated from its Fe (III)-transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (III) to Fe (II). The chromogenic agent, Ferene, form a highly colored Fe (II) - complex that measured photo metrically at 560nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe (II) iron to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe (II) ions are reacted with Ferrozine to form the color complex, which measured photo metrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding. The TIBC is determined by adding the serum iron value to the UIBC value.

Procedure: All the tubes/ cuvettes for Blank, Standard, Control and Samples were labeled.2.5ml of iron buffer reagent was added to all the tubes. Then 500µl of sample was added to respective tubes and mixed. 500µl of iron- free water was added to blank. Spectrophotometer was zeroed at 560nm with the reagent blank. The absorbance’s of all the tubes were read and recorded (A1 reading). Then 50µl of iron color reagent was added to all the tubes and mixed. All the tubes were placed in the heating bath.
at 37ºc for 10 minutes. Spectrophotometer was zeroed at 560nm with the reagent blank. The absorbance’s of all the tubes were read and recorded (A2 reading).

**Calculation**

$$A = \text{Absorbance}_{\text{Std}} - \text{Absorbance}_{\text{Test}}$$

$$\frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ Std} - A_1 \text{ Std}} \times \text{Conc. of Std} = \text{Total Iron (µg/dl)}$$

**2.1 Unsaturated Iron Binding Capacity (UIBC)**

**Procedure**: Two mls of UIBC buffer reagent was added to all tubes. 1.0ml of iron-free water was added to blank and mixed. To Standard, 500µl of iron-free water and 500µl of standard were added and mixed. To Test, 500µl of sample plus 500µl of iron standard were added and mixed. Spectrophotometer was zeroed at 560nm with reagent blank. The absorbance’s of all the tubes were read and recorded (A1 reading). 50µl of Iron color reagent was also added to all tubes and mixed. The tubes were placed in a heating bath at 37ºc for 10 minutes. Spectrophotometer was zeroed at 560nm with the reagent blank. The absorbance of all the tubes were read and recorded (A2 reading).

**UIBC calculation**

$$\text{Conc. of Std} - \frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ Std} - A_1 \text{ Std}} \times \text{Conc. of Std} = \text{UIBC (µg/dl)}$$

**2.2 Serum Ferritin (Enzyme Linked Immunosorbent Assay Method)**

**Principle**: The ferritin quantitative test is based on a solid phase enzyme linked immunosorbsent assay (ELISA). The assay system utilizes one rabbit anti- ferritin antibody for solid phase (microtiter wells) immobilization and mouse monoclonal anti-ferritin antibody in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme linked antibodies. After 45 minute incubation at room temperature, the wells are washed with water to remove unbounded labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of ferritin is directly proportional to color intensity of the test sample.

**Procedure**: Twenty microlitre (20µl) of standard, specimens and controls were added into appropriate wells. 100µl of enzyme conjugate reagent was dispensed into each well and gently mixed for 30 seconds. Then incubated at room temperature for 45 minutes. Incubated mixture was removed by flicking plate contents into sink. Microtiter wells were rinsed and flicked 5 times with distilled water. Wells were stroked sharply onto absorbent paper. 100µl of TMB reagent was dispensed into each well and was gently mixed for 10 seconds. Then incubated at room temperature in dark for 20 minutes. Reaction was stopped by addition of 100µl of stop solution into each well and was gently mixed for 30 seconds. When all the blue color changed to yellow color then the optical density was read at 450nm with a micro titer plate reader within 15 minutes.
Transferrin saturation percentage was calculated from the serum iron concentration and TIBC values as follows: transferrin saturation percentage = serum iron/TIBC x 100. Hemoglobin results were determined photo metrically using the HemoCue meter (Hb 301).

3 Statistics

The group comparisons were determined by one-way analysis of variance (ANOVA). Comparisons between mean values of iron parameters were compared with student’s t test. P value less than 0.05 was considered statistically significant.

4 Results

A total of 60 female students schooling in Enugu participated in this study. Only female student with hemoglobin levels of ≥ 12.0 g/dL were eligible for the study. The blood donors were grouped according to the number of units of blood they had given in one year. The 1st group (W1), (n=19), with mean age of 23 ± 1 year were apparently healthy female students with no previous history of blood donation. Donors in 2nd group (W2), (n=29), with mean age of 23 ± 3 years had given one unit of blood in one year. 3rd group (W3), (n=12), with mean age of 23 ± 3 years had donated two units of blood in one year. Ferritin, serum iron concentration, TIBC and transferrin saturation percentage were evaluated in all the three groups. The mean serum ferritin, serum iron, TIBC and percentage transferrin saturation compared among W1, W2 & W3 showed no significant difference (p>0.05) [Table 1]. The mean hemoglobin levels compared among W1, W2 & W3 showed significant difference (p<0.05).Iron depletion was found in 3.4% (1/29) in female donors that donated once in a year (W2). Iron deficiency was found in 3.4% of W2 and 8.3% (1/12) of W3 i.e female donors that have donated twice in one year [Table 2].

Table 1: Mean ±std of the iron parameters, Hb and age of the blood donors

<table>
<thead>
<tr>
<th>Groups</th>
<th>ferritin</th>
<th>serum iron</th>
<th>TIBC</th>
<th>%ts</th>
<th>Hb</th>
<th>age</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1(N=19)</td>
<td>71.32±35.01</td>
<td>21.63±10.82</td>
<td>80.58±14.81</td>
<td>25.58±10.81</td>
<td>13.92±0.43</td>
<td>23.32±0.89</td>
</tr>
<tr>
<td>W2(N=29)</td>
<td>56.28±38.23</td>
<td>20.93±18.11</td>
<td>85.17±23.20</td>
<td>20.93±12.33</td>
<td>13.20±0.90</td>
<td>23.38±2.83</td>
</tr>
<tr>
<td>W3 (N=12)</td>
<td>76.36±44.28</td>
<td>21.67±17.77</td>
<td>84.25±15.47</td>
<td>20.00±12.09</td>
<td>12.96±0.88</td>
<td>22.67±2.50</td>
</tr>
<tr>
<td>F(P)(value)</td>
<td>0.97(0.39)</td>
<td>0.02(0.99)</td>
<td>0.33(0.72)</td>
<td>1.15(0.32)</td>
<td>7.04(0.00)</td>
<td>0.43(0.66)</td>
</tr>
</tbody>
</table>

W1 VS W2 0.35 0.99 0.68 0.36 0.00*
W1 VS W3 0.62 1.00 0.79 0.41 0.01*
W2 VS W3 0.10 0.99 0.10 0.97 0.70*
Table 2: Distribution of donors according to iron stores

<table>
<thead>
<tr>
<th>Group</th>
<th>NDs</th>
<th>ID (F&lt;20ng/m)</th>
<th>IDY (F&lt;20ng/mL + %TS&lt;15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W2</td>
<td>29</td>
<td>3.4%</td>
<td>3.4%</td>
</tr>
<tr>
<td>W3</td>
<td>12</td>
<td>8.3%</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations

NDS = Number of donors
ID = Iron depletion
IDY = Iron deficiency
F = Ferritin
TS = Transferrin saturation
Std = Standard

5 Discussion

The present study showed that 8.3% of voluntary female blood donors in Enugu that donated twice in one year developed iron deficiency. In comparison with study [7] from Morocco which reported iron deficiency in 14% first time female blood donors, our obtained figures are lower in the present study. The reason behind our lower figure may be because the donors are educated individuals who take appropriate care of their health. Iron depletion was seen in 3.4% of first time donors. When compared with the same study done in Morocco which reported reduced iron stores in 19% first time female blood donors, our obtained figures are still lower in the present study. The mean age in this study is below the age reported of [8]. The hemoglobin levels in this study were slightly lower than that reported by Yousefinejad et al [9], though hemoglobin levels alone do not determine the iron storage. Serum ferritin concentration is generally accepted indicator for iron store. In this study, no significant difference were found between the first time donors and second time donors or their controls, this finding differed from other studies [7,10-14].

6 Conclusion

This study showed that female blood donors in Enugu develop iron deficiency and iron depletion when they donate once or twice in one year. This study also reveals that only university single female students can donate blood among others such as women civil/public servants, female health workers, market women and self employed women.

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References


