

## Original Article

# Iron status of deferred female blood donors due to low hemoglobin

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### Abstract:

**Introduction:** Regular blood donation leads to an iron loss in blood donors. Each time, donating 350-450 mL of blood will lose about 200-250 mg or 30% of the body's iron stores (BIS). Suppose the blood donor does not take iron supplements, affecting the red blood cell production and may develop iron deficiency anemia (IDA) in severe cases. **Objective:** This study assesses regular blood donors' hematological parameters and serum ferritin.

**Materials and Methods:** Six mL of CAT serum clot activator and K3-EDTA were collected from 40 regular blood donors. Specimens were divided into two groups of frequency of donation during June to October 2021; 1) 2-9 times and 2) more than 9 times. Progression of iron deficiency was divided into four stages; 1) serum ferritin level higher than 30 ng/mL for stage 0, 15 to 30 ng/mL for stage 1, lower than 15 ng/mL with normal Hb for stage 2, and lower than 15 ng/mL with Hb lower than 12.5 g/dL or anemia for stage 3 were evaluated. **Results:** The median age of total voluntary blood donors was 41. There was a 31% decrement serum ferritin in stage 0 between group 1 (75.72 ng/mL) compared with group 2 (52.25 ng/mL) and 0.4% decrement in stage 3 between group 1 (9.53 ng/mL) compared with group 2 (9.49 ng/mL). Of the blood donors, 45% were in Stage 3 of iron deficiency. Further donations in such cases may affect blood donor safety. **Conclusion:** Forty five percent of regular blood donors are in the IDA stage. After the first donation, initial screening for serum ferritin might help detect iron-deficient individuals. To Prevent this, iron supplementation is required after each blood donation.

**Keywords :** ● Regular blood donation ● Iron status ● Serum ferritin ● Iron supplementation

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## นิพนธ์ต้นฉบับ

# ระดับของธาตุเหล็กในผู้บริจาคโลหิตหญิงที่ไม่สามารถบริจาคโลหิตได้เนื่องจากมีค่าฮีโมโกลบินต่ำ

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<sup>1</sup>ห้องปฏิบัติการธนาคารเลือด โรงพยาบาลมหาราชนครศรีธรรมราช <sup>2</sup>สาขาวิชาเทคนิคการแพทย์ สำนักวิชาสหเวชศาสตร์ <sup>3</sup>ศูนย์วิจัยโลหิตวิทยาและวิทยาศาสตร์การบริการโลหิต มหาวิทยาลัยวลัยลักษณ์

## บทคัดย่อ

**บทนำ** การบริจาคโลหิตเป็นประจำทำให้ผู้บริจาคโลหิตสูญเสียธาตุเหล็ก โดยแต่ละครั้งของการบริจาคโลหิต 350-450 มล. จะสูญเสียธาตุเหล็กในร่างกายประมาณ 200-250 มก. หากผู้บริจาคโลหิตไม่ได้รับประทานธาตุเหล็กเสริมเพื่อสร้างเม็ดเลือดแดง อาจทำให้เกิดภาวะโลหิตจางจากการขาดธาตุเหล็กได้ **วัตถุประสงค์** ประเมินค่าพารามิเตอร์ทางโลหิตวิทยาและซีรั่มเฟอร์ริตินของผู้บริจาคโลหิตเป็นประจำ **วัสดุและวิธีการ** ตัวอย่างทดสอบในสารกันเลือดแข็งชนิด CAT serum clot activator และ K3-EDTA จำนวน 6 มล. จากผู้บริจาคโลหิตเป็นประจำ 40 ราย แบ่งเป็น 2 กลุ่มตามความถี่ของการบริจาคโลหิตในระหว่างเดือนมิถุนายนถึงเดือนตุลาคม พ.ศ. 2564 กลุ่มที่ 1 คือ ผู้บริจาคโลหิต 2-9 ครั้ง และกลุ่มที่ 2 คือ ผู้บริจาคโลหิตมากกว่า 9 ครั้ง นอกจากนี้ยังประเมินความรุนแรงของการขาดธาตุเหล็ก โดยแบ่งเป็น 4 ระยะ เริ่มจากระยะ 0 คือ ผู้บริจาคโลหิตมีระดับเฟอร์ริตินในซีรั่มสูงกว่า 30 ng/mL ระยะที่ 1 คือ ระดับเฟอร์ริติน 15 ถึง 30 ng/mL ระยะที่ 2 คือ ระดับเฟอร์ริตินต่ำกว่า 15 ng/mL ร่วมกับ Hb ปกติ และระยะที่ 3 คือ ระดับเฟอร์ริตินต่ำกว่า 15 ng/mL ร่วมกับ Hb ต่ำกว่า 12.5 g/dL หรือเป็นภาวะโลหิตจาง **ผลการศึกษา** อายุเฉลี่ยของผู้บริจาคโลหิต คือ 41 ปี เมื่อเปรียบเทียบระดับเฟอร์ริตินในซีรั่มพบว่าลดลงร้อยละ 31 ในผู้บริจาคโลหิตระยะ 0 ของกลุ่มที่ 1 (75.72 ng/mL) เปรียบเทียบกับกลุ่มที่ 2 (52.25 ng/mL) และลดลงร้อยละ 0.4 ในระยะที่ 3 ของกลุ่มที่ 1 (9.53 ng/mL) เปรียบเทียบกับกลุ่มที่ 2 (9.49 ng/mL) และพบว่าร้อยละ 45 ของผู้บริจาคโลหิตทั้งหมดมีความรุนแรงของการขาดธาตุเหล็กอยู่ในระยะที่ 3 แสดงให้เห็นว่าการบริจาคโลหิตอย่างต่อเนื่องอาจส่งผลกระทบต่อความปลอดภัยของผู้บริจาคโลหิต **สรุป** ร้อยละ 45 ของผู้บริจาคโลหิตเป็นประจำมีภาวะโลหิตจางจากการขาดธาตุเหล็ก ดังนั้น หลังการบริจาคโลหิตครั้งแรกควรตรวจคัดกรองซีรั่มเฟอร์ริตินในเลือดผู้บริจาคโลหิต เพื่อคัดกรองภาวะขาดธาตุเหล็ก อีกทั้งผู้บริจาคโลหิตทุกรายจำเป็นต้องรับประทานธาตุเหล็กเสริมหลังจากการบริจาคโลหิตทุกครั้ง

**คำสำคัญ :** ● บริจาคโลหิตเป็นประจำ ● ระดับของธาตุเหล็ก ● ซีรั่มเฟอร์ริติน ● ธาตุเหล็กเสริม

**วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2565;32:291-7.**

## Introduction

One of the most common causes of iron loss in blood donors is regular blood donation. Each time, donating 350-450 mL of blood loses about 200-250 mg or 30% of the body iron stores (BIS). Assuming that the donor does not take iron supplements, there is no iron to replenish the iron loss by blood donation, impairing erythropoiesis, and in severe cases resulting in iron deficiency anemia (IDA).<sup>1</sup>

IDA is the most common cause of anemia worldwide. It results in microcytic and hypochromic red blood cells on the peripheral blood smear. Patients often have fatigue and have difficult breathing with exertion. The causes of IDA differ depending on the person's age, gender, and socioeconomic position. It can be caused by a lack of iron, reduced adsorption, or blood loss, particularly in the elderly. Low dietary intake, higher systemic iron requirements, such as during pregnancy, and poor iron adsorption in celiac disease can all be the cause.<sup>2</sup> Blood tests, especially complete blood count (CBC) are used to diagnose IDA. Additional tests to assess serum ferritin, iron, total iron-binding capacity, or transferrin may be ordered.<sup>3</sup> Treatment of IDA is taking iron supplements and treating the underlying cause of iron deficiency if iron supplements do not increase blood iron levels. The role of iron supplementation is to replace iron storage and increase erythropoietin and oxygen transport within the body. Iron transport occurs through divalent metal transporter 1 (DMT1) across the cell membrane, combined and stored as ferritin in macrophages. This form is then converted to adsorbable Fe<sup>2+</sup> ions and then broken down by transferrin to various sites in the body, including the bone marrow for the erythropoiesis. Iron combines with other components such as porphyrin and globin chains to form hemoglobin, which transports oxygen from the lungs to other organs in the body.<sup>4,5</sup>

Blood donating on a regular basis can cause IDA and reduce the number of blood donors. As a result, the goal of this study is to evaluate the hematological characteristics and serum ferritin of regular blood donors,

as well as the risks of regular blood donors from IDA, so that appropriate care may be planned.

## Materials and Methods

### Specimen Collection

Forty regular blood donors, 37 females and 3 males, had answered a pre-donation donor questionnaire at the blood bank, Maharaj Nakhon Si Thammarat Hospital, Thailand, from June to October 2021. Six mL of blood were collected using CAT serum clot activator and K3-EDTA from all donors. The study has been approved by the Human Research Ethics Committee of Walailak University, Thailand (WUEC-21-054-01).

### Inclusion Criteria

Regular blood donors who donate blood more than or equal to 2 or more times and the hemoglobin level does not reach the criteria (normal hemoglobin value of 12.5 g/dL in females and 13.0 g/dL in males).<sup>6</sup> The selection of blood donors to participate in the study was randomized by purposive sampling within a time limit of 5 months. A total of 40 regular blood donors were obtained.

### Exclusion Criteria

Donors donated less than two times or healthy donors with hemoglobin levels in the normal value.

### Blood Tests

Hematological parameters; hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW) were analyzed by BECKMAN COULTER UniCel DxH 800 Coulter Cellular Analysis System (California, United States). Serum ferritin was examined by Cobas e 601 (Basel, Switzerland).

### Statistical Analysis

Test results were analyzed using SPSS version 20 (IBM, Chicago, IL, USA). Mean values of CBC and serum ferritin were analyzed with standard deviation and percentage for quantitative data. Data were analyzed using the one-sample Kolmogorov-Smirnov test for spec-

imen distribution. Differentiation between groups was analyzed using the Kruskal-Wallis H test and Post Hoc Multiple Comparisons, of which  $p \leq 0.05$  was considered statistically significant.

### Results

A total of 40 regular blood donors was recruited in this study. The median age is approximately 39 years. All donors were divided into two groups according to the frequency of blood donations; 2-9 times for group 1 and > 9 times for group 2. Group 1 consisted of 22 donors (55%), two males and 20 females, while group 2 consisted of 18 donors (45%), one male and 17 females (Table 1).

All donor blood specimens were tested for the hematological parameters, including Hb, Hct, MCV, MCH,

MCHC, and RDW and serum ferritin that measures the amount of iron stored in the body. The results showed no statistically significant differences in all parameters compared between groups 1 and 2. However, the data showed a downward trend in MCV, MCH, and MCHC in group 2 compared to group 1. MCV, MCH, and MCHC in group 1 were 81.94 fL, 26.71 pg/cell, and 32.55 g/dL, respectively. While MCV, MCH, and MCHC in group 2 were 79.60 fL, 25.63 pg/cell, and 32.16 g/dL, respectively (Table 2). Serum ferritin also showed a downward trend in group 2 compared to group 1 (Figure 1).

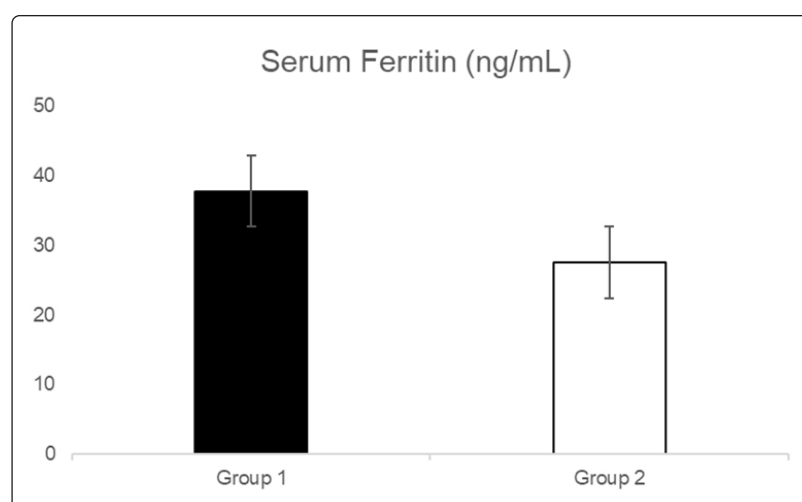
Considering the serum ferritin level and divided into four stages according to the severity of iron deficiency, serum ferritin level higher than 30 ng/mL for stage 0, 15 to 30 ng/mL for stage 1, lower than 15 ng/mL with normal Hb for stage 2, and lower than 15 ng/mL with

**Table 1** Number and age of regular blood donors

| Group<br>(frequency of donations) | Donor (male: female) | Percentage (%) | Age (years) (min - max) |
|-----------------------------------|----------------------|----------------|-------------------------|
| Group 1 (2-9 times)               | 22 (2:20)            | 55             | 38 (20-53)              |
| Group 2 (> 9 times)               | 18 (1:17)            | 45             | 41 (20-59)              |
| Total                             | 40                   | 100            | 41 (20-59)              |

**Table 2** Hematological parameters in regular blood donors

| Group          | Hb (g/dL)  | Hct (%)    | MCV (fL)   | MCH (pg/cell) | MCHC (g/dL) | RDW (%)    |
|----------------|------------|------------|------------|---------------|-------------|------------|
| Group 1        | 11.66±0.81 | 35.81±2.00 | 81.94±8.12 | 26.71±3.14    | 32.55±0.86  | 15.32±1.81 |
| Group 2        | 11.78±0.61 | 36.62±1.46 | 79.60±7.65 | 25.63±2.91    | 32.16±0.78  | 15.54±1.80 |
| ( $p < 0.05$ ) | 0.37       | 0.05       | 0.24       | 0.21          | 0.12        | 0.64       |



**Figure 1** Serum ferritin in regular blood donors (Group 1, 2-9 and Group 2, > 9 donations)

**Table 3** Serum ferritin and the severity of iron deficiency in regular blood donors

| Group            | Serum Ferritin (ng/mL) |            |         |           |
|------------------|------------------------|------------|---------|-----------|
|                  | Stage 0                | Stage 1    | Stage 2 | Stage 3   |
| Group 1 (n = 22) | 75.72±32.99            | 18.01±2.09 | -       | 9.53±2.42 |
| Group 2 (n = 18) | 52.25±35.30            | 26.56±2.22 | -       | 9.49±3.79 |
| p < 0.05         | 0.18                   | 0.06       | -       | 0.90      |

**Table 4** Distribution of serum ferritin and the severity of iron deficiency in regular blood donors

| Stages of iron deficiency                                         | Donor (n, %)  |
|-------------------------------------------------------------------|---------------|
| Stage 1 (serum ferritin 15-30 ng/mL)                              | 7 (17.5%)     |
| Stage 2 (serum ferritin < 15 ng/mL with normal Hb)                | 0 (0%)        |
| Stage 3 (serum ferritin < 15 ng/mL with Hb < 12.5 g/dL or anemia) | 18 (45%)      |
| Total                                                             | 25/40 (62.5%) |

Hb < 12.5 g/dL or anemia for stage 3, the results showed no statistically significant differences in serum ferritin level at all stages compared between groups 1 and 2. However, the data showed a downward trend in stage 0, and stage 3 of group 2, which is the number of donors and the serum ferritin level were decreased when compared with group 1. The number of donors at stage 0 of group 1 was 40.91% (n = 9), and group 2 was 33.33% (n = 6). While the number of donors at stage 3 of group 1 was 45.45% (n = 10), and group 2 was 44.44% (n = 8). The serum ferritin level at stage 0 of group 1 was 75.72 ng/mL, and group 2 was 52.25 ng/mL. While the serum ferritin level at stage 3 of group 1 was 9.53 ng/mL, and group 2 was 9.49 ng/mL (Table 3). The results showed that the distribution of the donors was 17.5% in stage 1 and 45% in stage 3 (Table 4).

### Discussion

Blood donor loses about 470 mL of blood volume and 200–250 mg of iron during a general whole blood donation. The median age of voluntary blood donors in this study was around 39 years old, which is between 17 and 70 years old, indicating a healthy age range for blood donation. The number of blood donors in groups 2 (45%) and 1 (55%) declined, especially male blood donors, in all two groups as compared to female blood donors; 2 and 1 males in groups 1 and 2, respectively.

Many factors might trigger an increase or decrease in blood donations, including vasovagal responses (VVRs), weakness, nausea, sweating, loss of consciousness, and seizures.<sup>7</sup> The reasons for a decrease in blood donations may include donors are dissatisfied with the service, injured, lack of motivation, unaware of the benefits of donating blood, or unable to donate blood. Population of Nakhon Si Thammarat, Thailand is 98.33 males per 100 females, so male blood donors were less than female blood donors.<sup>8</sup>

MCV, MCH, MCHC, and serum ferritin were among the hematological parameters that were found to be lower in regular blood donors. MCV analyzes the size of red blood cells. If blood cells are too small or too large, it may indicate a blood disorder. If the red blood cells are smaller than usual, it may indicate iron deficiency anemia (IDA), the most common form of anemia or thalassemia.<sup>9</sup> MCH shows the average amount of hemoglobin per red blood cell.<sup>10</sup> Low MCH indicates that red blood cells have less Hb than usual, implying that red blood cells are smaller than usual (microcytic). Iron insufficiency is one of the most common causes of low MCH<sup>11</sup> and thalassemia.<sup>12</sup> MCHC shows the average concentration of Hb per red blood cell.<sup>10</sup> Hypochromic microcytic anemia is the most common cause of low MCHC. Lack of iron, inability of the body to adsorb iron (celiac disease, Crohn's disease, and gastric bypass

surgery), chronic low-grade blood loss over time from a long menstrual cycle or peptic ulcers, and hemolysis are all possible causes of microcytic anemia.<sup>13</sup> Serum ferritin test determines the body's iron storage level. If ferritin level is lower than normal, it means the body's iron stores are depleted, and may have iron deficiency or be anemic. Hemochromatosis, porphyria, rheumatoid arthritis or other chronic inflammatory disorder, liver disease, hyperthyroidism, leukemia, Hodgkin's lymphoma, multiple blood transfusions, alcohol misuse, and taking too many iron supplements are all disorders that can cause elevated ferritin levels.<sup>14</sup> However, the results couldn't find any significant relationship between serum ferritin level and hematological parameters. This may be due to the interference factors from acute and chronic inflammation diseases affecting ferritin levels did not rule out. Ferritin is elevated in a wide range of inflammatory conditions, including chronic kidney disease, rheumatoid arthritis, and other autoimmune disorders, acute infections, and cancer.<sup>15</sup> That is why the serum ferritin showed an upward trend in stage 1 of group 2 ( $n = 3$ ) when compared to group 1 ( $n = 3$ ). Iron storage is reduced, and the severity of iron insufficiency increases, particularly in people who donate blood frequently, which could interfere with normal erythropoiesis.<sup>16</sup> Anemia is caused by a decrease in body iron stores. Iron is required for blood production. About 70% of the body's iron is found in Hb and myoglobin (Mb) in red blood cells and muscle cells, respectively. Hb is required for the delivery of oxygen from the lungs to the blood's tissues. Mb adsorbs, transports, and releases oxygen in muscle cells. About 6% of the body's iron is found in proteins that are required for respiration and energy metabolism, as well as enzymes involved in the synthesis of collagen and neurotransmitters. Iron is also required for healthy immunological function.

The body stores around 25% of ferritin, which is found in cells and blood. The average adult male possesses 1,000 mg of stored iron, which is sufficient for around three years, compared to only 300 mg for women (sufficient for about six months). Chronically low iron

intake can cause stores to run low and lower hemoglobin levels. Iron depletion is the state that results from the use up of all available iron. Iron deficiency anemia is the result of subsequent declines, which are referred to as IDA. The most prevalent cause of iron deficiency is blood loss.<sup>17</sup> Iron deficiency is almost always caused by gastrointestinal bleeding in men and postmenopausal women. Genitourinary blood loss frequently explains the increased need for iron in menstrual women. In contrast to intrauterine devices, which tend to increase menstrual bleeding, oral contraceptives tend to reduce menstrual blood loss. The need for iron is also increased by other genitourinary and respiratory tract bleeding factors. Blood donors lose 200 to 250 mg of iron with each donation. The amount of iron needed during growth spurts in infancy, youth, and adolescence may exceed the amount of iron available from diet and storage. Approximately 740 mg of iron are lost due to postpartum or postnatal bleeding, postpartum bleeding from delivery, and pregnancy-related tissue growth. The daily requirement for iron rises by roughly 0.5 to 1 mg during nursing.<sup>17</sup>

The limitation of this study was the small sample size, increasing the sample size and provide equal numbers of male and female blood donors for further study. It will help the study results to be more reliable.

### Conclusion

Regular blood donation was associated with a decrease in MCV, MCH, MCHC, and serum ferritin. Serum ferritin test can indirectly measure how much iron is in the body and evaluate the production of healthy red blood cells and the distribution of oxygen throughout the body. It is for the safety of blood donors to prevent IDA and blood product quality for the benefit of transfusion patients.

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