

## Assessment of Serum Ferritin and Hemoglobin Concentrations for Detecting Iron Deficiency in Females Aged 15–65 Years: A Cross-Sectional Study

Ghada Otman<sup>1</sup>, Mahbuba. M.Hassan<sup>2</sup>, Fatma Benkail<sup>3</sup>, Amal Elhassad<sup>4\*</sup>

<sup>1</sup> Higher Institute of Medical Sciences and Technologies, Al Bayda, Libya

<sup>2,3,4</sup> Laboratory Medicine Department, College of Medical Technology, Derna, Libya

تقييم تراكيز فيريتين السيروم والهيموجلوبين للكشف عن عوز الحديد لدى الإناث تتراوح أعمارهن بين 15 و65 عاماً: دراسة مقطعية

غادة عثمان<sup>1</sup>، محبوبة حسان<sup>2</sup>، فاطمة بن خيال<sup>3</sup>، أمل الحصادي<sup>4\*</sup>

<sup>1</sup> المعهد العالي للعلوم والتقنيات الطبية، البيضاء، ليبيا

<sup>2,3,4</sup> قسم طب المختبرات، كلية التقنية الطبية، درنة، ليبيا

\*Corresponding author: [elhassade.amal@gmail.com](mailto:elhassade.amal@gmail.com)

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### Abstract:

Background: Iron deficiency and anemia represent significant global public health challenges, particularly among females, necessitating precise biochemical and hematological evaluation. This cross-sectional study aimed to assess hemoglobin and serum ferritin concentrations and examine their interrelationships among 67 apparently healthy females aged 15–65 years, enrolled between January and April 2024. Methods: Participants with systemic infections, positive C-reactive protein levels, known chronic diseases, or recent iron and nutritional supplementation were excluded. Hemoglobin concentrations were quantified using an automated hematology analyzer, whereas serum ferritin levels were measured via the ichroma™ fluorescence immunoassay system. Anemia and iron deficiency were defined using established clinical thresholds. Results: The mean participant age was  $31.58 \pm 16.66$  years, with mean hemoglobin and ferritin concentrations of  $11.56 \pm 1.49$  g/dL and  $27.23 \pm 116.39$  µg/L, respectively. Low hemoglobin levels were identified in 58.2% of participants, while 43.3% exhibited depleted serum ferritin. A statistically significant difference in serum ferritin was observed between participants aged  $\leq 17$  years and those aged  $\geq 18$  years ( $P = 0.029$ ), whereas hemoglobin levels showed no significant age-related variation. A moderate negative correlation was detected between hemoglobin and serum ferritin concentrations ( $r = -0.435$ ,  $P = 0.004$ ). Conclusion: The findings demonstrate a high prevalence of low hemoglobin and depleted iron stores within this cohort, emphasizing the necessity of dual biomarker assessment. The unexpected inverse correlation warrants further multi-center investigation incorporating comprehensive inflammatory and iron-status markers.

**Keywords:** Iron deficiency; anemia; hemoglobin; serum ferritin; females; iron status.

## الملخص

الخلفية: تمثل عوز الحديد فقر الدم تحديات صحية عامة عالمية هامة، لا سيما بين الإناث، مما يستلزم تقييماً دقيقاً بيوكيميائياً ودماً. هدفت هذه الدراسة مقطعية المقطع إلى تقييم تراكيز الهيموجلوبين وفيريتين السيروم ودراسة ارتباطاتها المتبادلة بين 67 أنثى سليمة ظاهرياً تتراوح أعمارهن بين 15 و65 عاماً، تم تسجيلهن بين يناير وأبريل 2024. الطرق: تم استبعاد المشاركات اللاتي يعانين من التهابات جهازية، أو ارتفاع في بروتين التفاعل C ، أو أمراض مزمنة معروفة، أو اللاتي تناولن مكملات حديد أو تغذية مؤخراً. تم قياس تراكيز الهيموجلوبين باستخدام محلل دموي آلي، بينما قُلت مستويات فيريتين السيروم عبر نظام المقايسة المناعية الفلورية. ichroma<sup>TM</sup> تم تحديد فقر الدم وعوز الحديد باستخدام عتبات سريرية معتمدة. النتائج: بلغ متوسط أعمار المشاركات  $31.58 \pm 16.66$  عاماً، في حين بلغ متوسط تراكيز الهيموجلوبين والفيريتين على التوالي  $11.56 \pm 1.49$  جرام/ديسيلتر و  $27.23 \pm 116.39$  ميكروجرام/لتر. تم رصد انخفاض مستويات الهيموجلوبين لدى 58.2% من المشاركات، بينما أظهرت 43.3% استنفاداً في مخزون الفيريتين. لوحظ وجود فرق ذو دلالة إحصائية في فيريتين السيروم بين المشاركات اللاتي تبلغ أعمارهن 17 سنة فأقل واللاتي تبلغ أعمارهن 18 سنة فأكثر ( $P = 0.029$ ) ، بينما لم تُظهر تراكيز الهيموجلوبين تبايناً عمرياً ذا دلالة. وتم الكشف عن ارتباط سلبي متوسط بين تراكيز الهيموجلوبين وفيريتين السيروم ( $r = -0.435$ ،  $P = 0.004$ ). الاستنتاج: توضح النتائج انتشاراً مرتفعاً لانخفاض الهيموجلوبين ومخزون الحديد المستنفد ضمن هذه العينة، مما يؤكد على ضرورة التقييم المزدوج للمؤشرات الحيوية. يستدعي الارتباط العكسي غير المتوقع إجراء المزيد من الدراسات متعددة المراكز التي تدمج علامات التهابية وحالة حديدية شاملة.

**الكلمات المفتاحية:** عوز الحديد؛ فقر الدم؛ الهيموجلوبين؛ فيريتين السيروم؛ الإناث؛ حالة الحديد..

## Introduction

Iron deficiency is one of the most common nutritional deficiencies worldwide and remains an important public health concern, particularly among females of reproductive age. Iron is essential for hemoglobin synthesis, oxygen transport, cellular energy production, and normal physiological functions. When iron stores become depleted, iron deficiency may progress to iron deficiency anemia (IDA), although iron deficiency can also occur before anemia develops (Pasricha et al., 2021; Al-Naseem et al., 2021).

Hemoglobin (Hb) concentration is widely used to detect and assess anemia; however, Hb alone cannot determine whether anemia is caused by iron deficiency because anemia may result from several nutritional, inflammatory, infectious, and chronic conditions (Pasricha et al., 2021). Therefore, assessment of iron stores is important when investigating suspected iron deficiency. Serum ferritin is a widely used marker of body iron stores. Low ferritin concentrations generally indicate depleted iron stores and may identify iron deficiency before a significant reduction in hemoglobin occurs (WHO, 2020a; Sholzberg et al., 2025). The World Health Organization recommends a ferritin concentration below 15 µg/L as an indicator of iron deficiency in apparently healthy adults (WHO, 2020a).

However, ferritin is also an acute-phase reactant and may increase in response to infection, inflammation, liver disease, and other conditions. Consequently, normal or elevated ferritin concentrations do not necessarily exclude iron deficiency when inflammation is present (Rohr et al., 2023; Mahroum et al., 2022). Recent studies have also suggested that higher ferritin thresholds, such as 30 µg/L, may improve the detection of iron deficiency in some populations (Jäger et al., 2024; Truong et al., 2024).

The combined assessment of hemoglobin and serum ferritin therefore provides complementary information: hemoglobin reflects the hematological consequence of iron deficiency, whereas

ferritin reflects iron storage. Evaluating both parameters may improve the identification of individuals with iron deficiency and iron deficiency anemia (Al-Naseem et al., 2021; Kriel et al., 2025).

Therefore, this study aims to assess serum ferritin and hemoglobin concentrations and to investigate the relationship between these parameters in the study population. The findings may contribute to a better understanding of iron status and the usefulness of these laboratory indicators for detecting iron deficiency anemia.

## **Materials and Methods**

### **Study Population and Sample Collection**

A total of 67 female participants aged 15–65 years were enrolled in the study between January 2024 and April 2024. The study samples and laboratory results were obtained from the Laboratory Medicine Clinic. Healthy women were included in the study. Participants were excluded if they were taking nutritional or iron supplements, had a known systemic disease or infection, or had a positive C-reactive protein (CRP) result, as inflammatory conditions may increase serum ferritin concentrations independently of iron status.

Written informed consent was obtained from all participants before sample collection. Approximately 5 mL of venous blood was collected from each participant for the assessment of hemoglobin and serum ferritin concentrations.

### **Blood Sample Collection and Preparation**

Venous blood samples were collected using standard aseptic procedures. Approximately 5 mL of blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for hematological analysis. The tubes were appropriately labeled, gently mixed to ensure adequate anticoagulation, and transferred immediately to the laboratory for analysis.

### **Laboratory Analysis**

#### ***Hemoglobin Estimation***

Complete blood count (CBC) analysis was performed on all 67 EDTA-anticoagulated blood samples using an automated hematology analyzer (Sysmex XK-21N, 2012, Germany). Hemoglobin concentration was measured as part of the CBC analysis.

Automated hematology analyzers provide rapid and standardized measurements of major hematological parameters. Depending on the analyzer, cellular measurements may be based on electrical impedance, optical measurement, or flow-cytometric principles. Hemoglobin concentration is generally determined using a photometric method following the lysis of red blood cells. Automated analyzers incorporate calibration and quality-control procedures to ensure the accuracy and reliability of laboratory measurements.

For this study, anemia was defined according to the World Health Organization (WHO) criterion as a hemoglobin concentration <12.0 g/dL in non-pregnant women aged 15–65 years.

#### ***Serum Ferritin Estimation***

Serum ferritin concentration was determined using the ichroma™ fluorescence immunoassay system based on a sandwich immunodetection principle. In this method, ferritin antigen present in the sample reacts with detector antibodies in the detection buffer to form antigen–antibody complexes. These complexes migrate through a nitrocellulose membrane and are captured by immobilized antibodies on the test cartridge. The intensity of the resulting fluorescence signal is proportional to the concentration of ferritin in the sample and is measured by the ichroma™ instrument.

### **Ferritin Test Procedure**

The ferritin assay was performed according to the manufacturer's instructions. Briefly, 150 µL of detector diluent was transferred into a detector tube containing a granule and mixed until the granule was completely dissolved to form the detection buffer. The detection buffer was used within the recommended time.

Subsequently, 30 µL of the participant's serum/plasma sample was transferred into the detector tube. The tube was closed and mixed thoroughly by shaking approximately 10 times. Then, 75 µL of the prepared sample mixture was transferred into the sample well of the test cartridge. The loaded cartridge was incubated in the i-Chamber or an incubator at approximately 25°C for 10 minutes. Following incubation, the cartridge was inserted into the cartridge holder of the ichroma™ analyzer in the correct orientation, and the ferritin concentration was measured according to the manufacturer's instructions.

### Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), Version 26.0. Descriptive statistics were used to summarize the characteristics of the study participants. Continuous variables, including age, hemoglobin concentration, and serum ferritin concentration, were presented as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. According to WHO criteria, iron deficiency was defined as a serum ferritin concentration <15 µg/L (ng/mL). Iron-deficiency anemia (IDA) was defined as the simultaneous presence of anemia (hemoglobin <12.0 g/dL) and iron deficiency (serum ferritin <15 µg/L). Participants with serum ferritin <15 µg/L but hemoglobin ≥12.0 g/dL were classified as having iron deficiency without anemia.

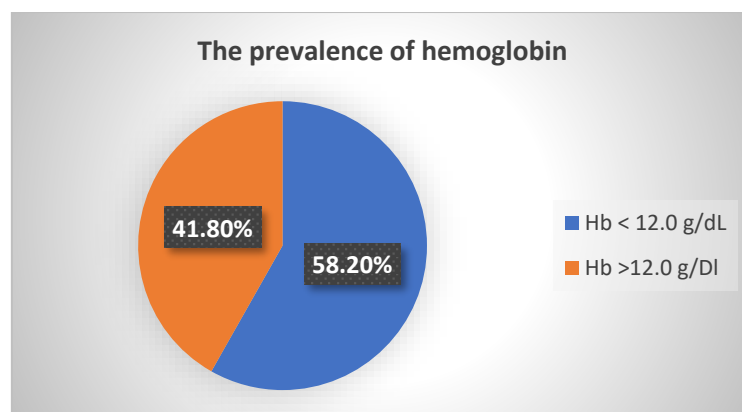
An independent-samples *t*-test was used to compare the mean age between participants with low and normal hemoglobin concentrations and between participants with low and normal serum ferritin concentrations. The independent-samples *t*-test was also used to compare mean hemoglobin and serum ferritin concentrations between the two age groups (≤17 and ≥18 years). The relationship between hemoglobin and serum ferritin concentrations was assessed using the Pearson correlation coefficient (*r*). A 95% confidence interval (CI) was used for prevalence estimates where appropriate. A *p*-value of <0.05 was considered statistically significant.

## Results

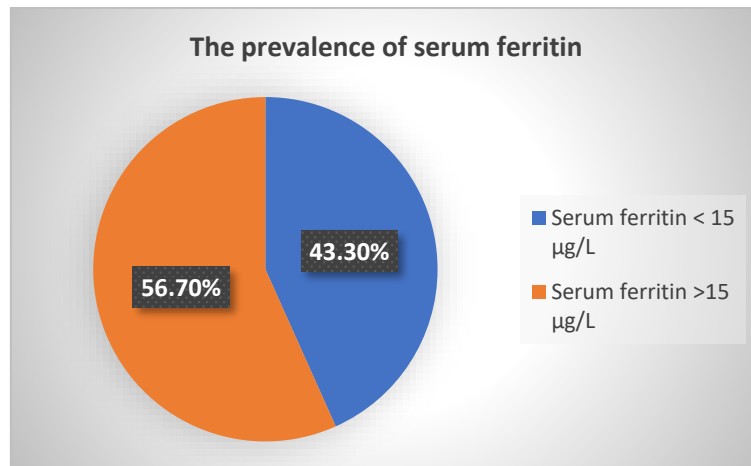
A total of 67 female participants were included in the study, with ages ranging from 4 to 65 years and a mean age of 31.58 ± 16.66 years. The mean hemoglobin concentration was 11.56 ± 1.49 g/dL. Using a cutoff value of 12.0 g/dL, 39 participants (58.2%) had hemoglobin concentrations below 12.0 g/dL, while 28 (41.8%) had concentrations ≥12.0 g/dL.

The mean serum ferritin concentration was 27.23 ± 116.39 µg/L. Based on a ferritin cutoff of 15 µg/L, 29 participants (43.3%) had serum ferritin concentrations below 15 µg/L, indicating low or depleted iron stores, whereas 38 (56.7%) had concentrations ≥15 µg/L. Overall, low hemoglobin was more frequently observed than low serum ferritin.

So, the prevalence of hemoglobin below 12.0 g/dL was 58.2% (95% CI: approximately 45.6%–69.7%)(Figure 1), while the prevalence of serum ferritin below 15 µg/L was 43.3% (95% CI: approximately 31.4%–55.2%)(Figure 2).



**Figure 1: Prevalence of hemoglobin**



**Figure 2:** Prevalence of serum ferritin

**Table 1:** Relationship Between Serum Ferritin Status and Age Among Female Participants

| Serum Ferritin Status   | Mean Age $\pm$ SD (years) | t-value | p-value |
|-------------------------|---------------------------|---------|---------|
| <15 µg/L (Low)          | 29.00 $\pm$ 15.50         | -1.110  | 0.271   |
| $\geq$ 15 µg/L (Normal) | 33.55 $\pm$ 17.50         |         |         |

The participants were divided into two groups according to their serum ferritin concentrations. The low-ferritin group, comprising 29 females with serum ferritin concentrations <15 µg/L, had a mean age of  $29.0 \pm 15.5$  years, while the normal-ferritin group, comprising 38 females with serum ferritin concentrations  $\geq 15$  µg/L, had a mean age of  $33.55 \pm 17.5$  years. An independent-samples *t*-test showed no statistically significant difference in mean age between the two groups ( $t = -1.110$ ,  $p = 0.271$ ). Therefore, age was not significantly associated with serum ferritin status in the study population (Table 1).

**Table 2:** Relationship Between Hemoglobin Status and Age Among Female Participants

| Hemoglobin Status            | Mean Age $\pm$ SD (years) | t-value | p-value |
|------------------------------|---------------------------|---------|---------|
| Hb <12.0 g/dL (Low)          | 28.38 $\pm$ 17.57         | -1.889  | 0.063   |
| Hb $\geq$ 12.0 g/dL (Normal) | 36.04 $\pm$ 14.50         |         |         |

The mean age was lower among participants with low hemoglobin concentrations ( $28.38 \pm 17.57$  years) than among those with hemoglobin concentrations  $\geq 12.0$  g/dL ( $36.04 \pm 14.50$  years). However, this difference was not statistically significant ( $t = -1.889$ ,  $p = 0.063$ ) (Table 2).

**Table 3:** Relationship Between Age Group and Hemoglobin and Serum Ferritin Concentrations

| Age Group (years) | Serum Ferritin Mean $\pm$ SD (µg/L) | t-value | p-value | Hemoglobin Mean $\pm$ SD (g/dL) | t-value | p-value |
|-------------------|-------------------------------------|---------|---------|---------------------------------|---------|---------|
| $\leq 17$         | 25.50 $\pm$ 36.24                   | -2.223  | 0.029   | 10.90 $\pm$ 2.20                | -1.766  | 0.082   |
| $\geq 18$         | 27.68 $\pm$ 31.34                   |         |         | 11.40 $\pm$ 1.30                |         |         |

The mean hemoglobin concentration was lower among participants aged  $\leq 17$  years ( $10.90 \pm 2.20$  g/dL) compared with those aged  $\geq 18$  years ( $11.40 \pm 1.30$  g/dL). However, the difference was not statistically significant ( $t = -1.766$ ,  $p = 0.082$ ).

Similarly, the mean serum ferritin concentration was  $25.50 \pm 36.24$   $\mu\text{g/L}$  among participants aged  $\leq 17$  years and  $27.68 \pm 31.34$   $\mu\text{g/L}$  among those aged  $\geq 18$  years. Although the reported analysis indicated statistically significant association between age group and serum ferritin concentration ( $t = -2.223$ ,  $p = 0.029$ ) (Table 3).

**Table 4:** Correlation Between Hemoglobin and Serum Ferritin Concentrations

| Variables                     | Pearson correlation (r) | p-value |
|-------------------------------|-------------------------|---------|
| Hemoglobin vs. Serum ferritin | -0.435**                | 0.004   |

The correlation between hemoglobin and serum ferritin is negative and statistically significant ( $r = -0.435$ ,  $p = 0.004$ ). This indicates a moderate negative correlation, meaning that higher hemoglobin values were associated with lower serum ferritin values in this sample (Table 4).

## Discussion

The present study assessed hemoglobin and serum ferritin concentrations among 67 female participants and examined their relationships with age and age group. The main findings were that 58.2% of participants had hemoglobin concentrations below 12.0 g/dL, while 43.3% had serum ferritin concentrations below 15  $\mu\text{g/L}$ . No statistically significant difference was observed in mean age between participants with low and normal ferritin status or between those with low and normal hemoglobin status. However, serum ferritin concentrations differed significantly between the two age groups, whereas hemoglobin concentrations did not. A statistically significant moderate negative correlation was also observed between hemoglobin and serum ferritin concentrations.

The prevalence of low hemoglobin in the present study was relatively high, with 58.2% of participants having hemoglobin concentrations below 12.0 g/dL. This finding indicates a considerable burden of anemia among the study participants. Iron deficiency is an important cause of anemia, particularly among females, although anemia may also result from other conditions, including chronic disease, infection, nutritional deficiencies, blood loss, and inherited disorders (Pasricha et al., 2021; Sholzberg et al., 2025). Therefore, the high prevalence of low hemoglobin observed in this study should not be interpreted as being entirely attributable to iron deficiency without additional investigations.

In comparison, 43.3% of participants had serum ferritin concentrations below 15  $\mu\text{g/L}$ . The lower proportion of participants with low ferritin compared with those with low hemoglobin suggests that not all participants with low hemoglobin necessarily had depleted iron stores according to the cutoff used in this study. This finding highlights the importance of assessing both hemoglobin and ferritin because hemoglobin reflects the hematological consequence of iron deficiency, whereas ferritin provides an indication of iron stores. Iron deficiency may also occur before the development of anemia, meaning that some individuals may have depleted iron stores despite having hemoglobin concentrations above the anemia threshold (Al-Naseem et al., 2021; WHO, 2020).

The ferritin cutoff of  $<15$   $\mu\text{g/L}$  used in this study was based on the WHO criterion for iron deficiency in apparently healthy individuals. However, recent studies have suggested that higher ferritin thresholds may identify additional individuals with clinically relevant iron deficiency. Jäger et al. (2024) reported that the prevalence of iron deficiency increased substantially when higher ferritin cutoffs were used. Similarly, recent clinical literature has suggested that ferritin concentrations below approximately 30  $\mu\text{g/L}$  may provide a more

sensitive indication of iron deficiency in some populations, particularly females (Sholzberg et al., 2025; Truong et al., 2024). Therefore, the prevalence of iron deficiency in the present study may have been higher if a higher ferritin threshold had been applied.

Regarding age, participants with low ferritin had a mean age of  $29.00 \pm 15.50$  years, compared with  $33.55 \pm 17.50$  years among participants with ferritin  $\geq 15$   $\mu\text{g/L}$ . This difference was not statistically significant ( $t = -1.110$ ,  $p = 0.271$ ). Similarly, participants with low hemoglobin had a mean age of  $28.38 \pm 17.57$  years compared with  $36.04 \pm 14.50$  years among those with hemoglobin  $\geq 12.0$  g/dL, although this difference also did not reach statistical significance ( $t = -1.889$ ,  $p = 0.063$ ). These findings suggest that when age was considered as a continuous variable, it was not significantly associated with either ferritin status or hemoglobin status.

However, a statistically significant difference in serum ferritin concentration was observed when participants were divided into younger ( $\leq 17$  years) and adult ( $\geq 18$  years) groups. The mean ferritin concentration was  $25.50 \pm 36.24$   $\mu\text{g/L}$  among participants aged  $\leq 17$  years and  $27.68 \pm 31.34$   $\mu\text{g/L}$  among those aged  $\geq 18$  years ( $t = -2.223$ ,  $p = 0.029$ ). Although the difference in mean values was relatively small, the statistical result indicates that ferritin concentrations differed significantly between the two age groups. This finding may reflect differences in physiological development, dietary requirements, menstrual status, or other age-related factors. Because ferritin is also affected by inflammation and other physiological conditions, age alone should not be considered the sole explanation for this difference (WHO, 2020; Rohr et al., 2023).

In contrast, hemoglobin concentration did not differ significantly between the two age groups. Participants aged  $\leq 17$  years had a lower mean hemoglobin concentration than those aged  $\geq 18$  years ( $10.90 \pm 2.20$  vs.  $11.40 \pm 1.30$  g/dL), but the difference was not statistically significant ( $t = -1.766$ ,  $p = 0.082$ ). The lack of statistical significance may partly be related to the relatively small sample size and variability in hemoglobin concentrations. Thus, age group was significantly associated with serum ferritin but not with hemoglobin concentration in this study. Another important finding was the statistically significant negative correlation between hemoglobin and serum ferritin concentrations ( $r = -0.435$ ,  $p = 0.004$ ). This represents a moderate inverse relationship, indicating that higher hemoglobin values were associated with lower ferritin values in the present sample. However, this finding should be interpreted cautiously because the direction of the association is not consistent with the expected physiological relationship between iron stores and hemoglobin. In iron deficiency, depletion of iron stores generally occurs before or alongside a reduction in hemoglobin. Therefore, a negative correlation may indicate the influence of other factors or extreme observations rather than a direct biological relationship.

The substantial variability in serum ferritin observed in the study should also be considered when interpreting this correlation. The mean ferritin concentration was  $27.23 \pm 116.39$   $\mu\text{g/L}$ , indicating a very large standard deviation relative to the mean. Such variability may suggest the presence of unusually high ferritin values or outliers that could substantially influence the Pearson correlation coefficient. Ferritin can also increase independently of iron stores in response to inflammation, infection, liver disease, metabolic conditions, and other pathological processes (Mahroum et al., 2022; Rohr et al., 2023). Therefore, the negative correlation should be confirmed by examining the scatterplot and checking the data for extreme values.

The findings of this study should be interpreted in light of several limitations. First, the sample size was relatively small, with only 67 participants, which may limit the generalizability of the findings and reduce statistical power for detecting some associations. Second, the study included females across a wide age range, including participants younger than 18 years and adults, which may introduce physiological differences between age groups. Third, additional indicators of iron status, such as transferrin saturation, soluble transferrin receptor, or total iron-binding capacity, were not assessed. Furthermore, although participants with inflammatory

conditions were excluded according to the study criteria, the availability of inflammatory biomarkers such as C-reactive protein would provide additional support for interpreting ferritin concentrations.

Despite these limitations, the present study provides useful information regarding hemoglobin and serum ferritin status among females. The relatively high prevalence of low hemoglobin and low ferritin emphasizes the importance of evaluating both hematological and biochemical indicators when investigating iron deficiency. The significant difference in ferritin between age groups also suggests that age-related factors may influence iron status and should be considered when interpreting ferritin concentrations.

In conclusion, 58.2% of the participants had hemoglobin concentrations below 12.0 g/dL, while 43.3% had serum ferritin concentrations below 15 µg/L. Age was not significantly associated with hemoglobin status or ferritin status when analyzed using continuous age; however, serum ferritin concentrations differed significantly between participants aged ≤17 and ≥18 years. Hemoglobin did not differ significantly between the two age groups. A significant negative correlation was observed between hemoglobin and ferritin, although this unexpected direction warrants cautious interpretation and further investigation of potential outliers and confounding factors. Larger studies including additional iron-status and inflammatory biomarkers are recommended to clarify the relationship between hemoglobin, ferritin, and age among females.

### **Compliance with ethical standards**

#### *Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

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