

## Evaluation Of Iron Level In Reproductive And Postmenopausal Women In Relation To Lifestyle

Ehsan H. Al-dabbagh<sup>1</sup> , Zainab M. Ali<sup>1</sup> , Mohammed KJ. Alnori<sup>2\*</sup>

<sup>1</sup>College of Medicine, University of Mosul, Mosul, Iraq

<sup>2</sup>College of Pharmacy, University of Mosul, Mosul, Iraq

---

### ABSTRACT

Iron is an essential element in the maintenance of cellular quasi-equilibrium and normal organs function. Deterioration of serum iron or iron-linked parameters jeopardizes the internal cellular and subcellular machinery affecting major organ function leaving great physical and mental impacts on affected patients. The present study aimed to evaluate the iron status in women considering routine daily parameters, such as age of participants, employability, and nutritional status. A total of 50 subjects were enrolled in the present study and blood was withdrawn from all of them for measurement of haemoglobin concentration and serum iron and iron-binding capacity quantification. The results confirmed that low nutritional status significantly reduced haemoglobin, serum iron, and iron-binding capacity. The result also approved that employability affects the percentage of transferrin saturation. In conclusion, routine daily activity and lifestyle unexpectedly modulated iron status, this should be considered for women who are planning for pregnancy. The outcome raises the recommendation of low-dose iron supplementation in affected target groups.

**Keywords:** Iron deficiency; anaemia; iron status; haemoglobin

---

### INTRODUCTION

The body iron intake in healthy individuals is concocting through intestinal absorption of dietary iron. Obligatory depletion accounts for the majority of iron deficiency levels in both males and females (1). Furthermore, menstrual cycle and pregnancy cause significant physiologic iron losses in women of child-bearing age which jeopardize the quasi-equilibrium of organs and tissues(2). Iron shortage (3) or iron excess (4) have detrimental effects on bodily functioning, impairing standard of living and longevity.

According to a recent assessment on a worldwide incidence of disease(2), 22.5 percent of non-pregnant girls aged 15 to 49 suffer anaemia, which is mostly related to iron deficiency. High physiological iron losses in women of childbearing age place stress on the absorption of iron, which is in consequence reliant on intake of iron, both quantitatively and qualitatively. According to a recent survey of bodily iron deficiency in European women of childbearing age (5), 40–55% had limited or nonexistent iron deficiency stores, defined as serum ferritin less than 30 g/L.

ID was found to be 10–32 percent common, while iron deficiency was shown to be 2–5% common. Around 20–35% of the women have adequate iron stores (serum ferritin >70 g/L) to be able to carry a pregnancy without any need for supplemental iron (5). The number of European females has poor iron levels, which may be related to a lack of iron stores, a lack of ferritin, and/or an imbalance in the concentration of stimulants and blockers of absorption of iron in the meal.

Anemia has been recognized as a separate potential cause for morbidity and death in several investigations, regardless of race or gender or age(6). Anemia in females might be linked to reduced cognitive performance, concentration, and awareness (in both women and their children)(7,8,9,10,11); lower preterm birth infants and a potentially higher likelihood of preterm birth; and disrupted postpartum nurturing interaction, which could lead to developmental deficits in children(12,13,14). In the present study, we quantified iron status and iron-related parameters based on two-dimension; socioeconomic status and nutritional quality.

#### **PATIENTS AND METHODS**

After consent approval by candidates; fifty healthy women have joined this study, 31 at postmenopausal and 19 at child-bearing age; mean age 39 and 58, respectively. Enrolled candidates were reclassified according to occupation whether employed or unemployed and according to nutrition state (poor, fair, or good), the duration of menopause in years, and hematinic intake those with menorrhagia were excluded depending on questioner forum. Blood samples were withdrawn from individual patients and divided into two fractions; anticoagulated whole blood sample for Hb% and coagulated blood sample for serum collection (to measure serum iron and total iron-binding capacity).

Haemoglobin was measured by Drabkin's method; the principle of the assay is that the haemoglobin is oxidized by potassium ferricyanide to methemoglobin in alkaline media, which

will then be transformed by potassium cyanide to cyanomethemoglobin. The intensity of the colour produced is proportional to the concentration of haemoglobin in the specimen. The procedure of assay involved using whole blood after mixing with the anticoagulant. We mixed 20µl of venous blood in the anticoagulated tube with 4ml of Drabkin's solution and read at 540nm using a spectrophotometer. A standard serial dilution was prepared to quantify the haemoglobin concentration in a subsequent step against blank Drabkin's solution from the standard calibration curve.

Serum iron concentrations were quantified using a kit supplied by Biolabo (92108, France). The principle of assay endowed that; ascorbic acid, after dissociating iron-transferrin coupled in acid media, lowers Fe<sup>+3</sup> to Fe<sup>+2</sup>, which forms a colourful complex with Ferene. The absorbance at 600 nm is dependent on the quantity of iron in the sample. To minimize copper interaction, thiourea is incorporated into the reaction mixture.

Total iron-binding capacity was determined using a kit supplied Biolabo (92308, France). According to the following procedure; after adding enough Fe<sup>+3</sup> to saturate transferrin, the surplus Fe<sup>+3</sup> is eliminated by absorption with basic magnesium carbonate powder, and the bound iron in the residue is determined after spinning.

The percentage of transferrin saturation was calculated using the equation below (where s. Fe is serum iron and TIBC is the total iron-binding capacity)

$$\text{The percentage of transferrin saturation} = \frac{100 \times s.\text{Fe}}{\text{TIBC}}$$

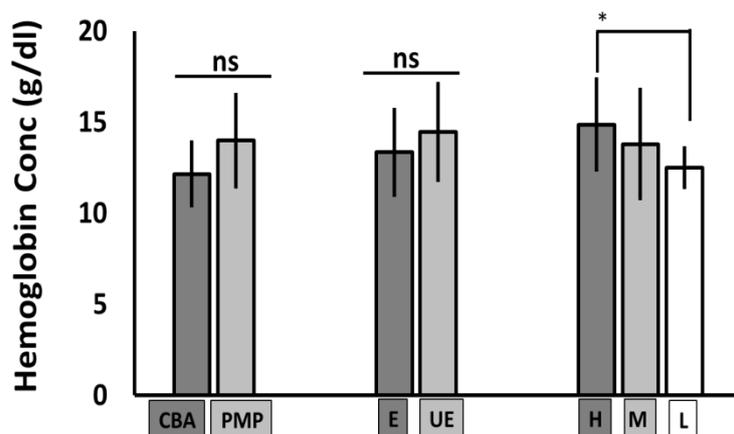
### **Statistical Analysis**

The statistical analysis was conducted on the results and results were represented in a histogram representing ad a mean (histogram column) and the error bars are standard deviations (Figures 1,2,3,4). The differences were considered significant when the P value is less than 0.05 (P<0.05). The analysis was conducted using statistical software Graphpad prism V.6 (USA) and excel spreadsheets.

### **RESULTS**

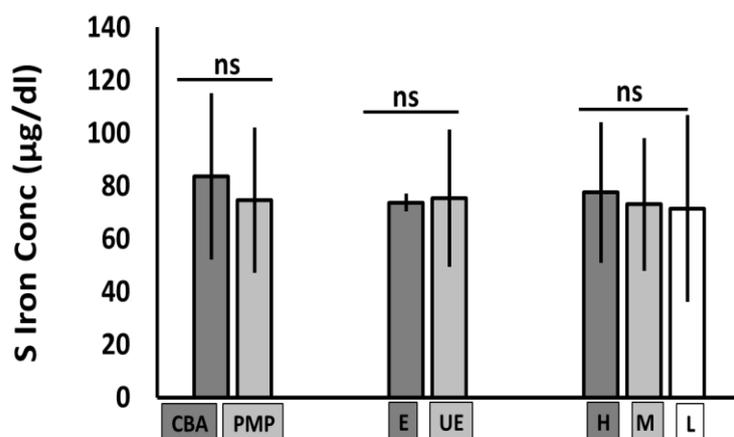
The result of the haemoglobin test has confirmed a non-significant (P>0.05) differences between child-bearing age and postmenopausal women (12.15±1.85 and 13.98±2.63, respectively) and non-significant (P>0.05) differences exist between employed and unemployed women (13.34±2.44 and 14.45±2.74, respectively). However, haemoglobin concentration in low nutritional status women (12.5±1.17) was significantly (P<0.05) lower

than high or moderate nutritional status ( $14.86 \pm 2.59$  and  $13.79 \pm 3.08$ , respectively), as shown in figure 1.



**Figure 1.** Low nutritional status has significantly lower haemoglobin concentration as an independent variable. Haemoglobin shows no change in association with age or employment status. Data expressed as mean $\pm$ SD, \*P<0.05 CBA=child-bearing age, PMP=postmenopausal, E=employed, UE=unemployed, H=high nutritional status, M=middle nutritional status, L=low nutritional status, ns=non-significant.

The result of the serum iron test has confirmed a non-significant ( $P > 0.05$ ) differences between child-bearing age and postmenopausal women ( $83.64 \pm 31.49$  and  $74.65 \pm 27.45$ , respectively) and non-significant ( $P > 0.05$ ) differences exist between employed and unemployed women ( $73.71 \pm 3.3$  and  $75.33 \pm 26.08$ , respectively). Moreover, non-significant ( $P > 0.05$ ) differences exist based on the nutritional status of women, whether high, moderate or low ( $77.48 \pm 26.63$ ,  $73.01 \pm 25.08$ , or  $71.36 \pm 35.36$ , respectively), as shown in figure 2.



**Figure 2.** Iron status shows no change in association with age or employment status or nutritional status. Data expressed as mean±SD. CBA= child-bearing age, PMP= postmenopausal, E=employed, UE=unemployed, H=high nutritional status, M=middle nutritional status, L=low nutritional status, ns=non-significant.

The result of percentage transferrin saturation has confirmed significant ( $P<0.05$ ) differences between child-bearing age and postmenopausal women ( $51.98\pm 25.35$  and  $34.8\pm 21.87$ , respectively) and significant ( $P<0.05$ ) differences exist between employed and unemployed women ( $28.8\pm 14.18$  and  $39.13\pm 25.59$ , respectively). Moreover, percentage transferrin saturation in low nutritional status women ( $27.6\pm 16.15$ ) was significantly ( $P<0.05$ ) lower than high nutritional status ( $35.14\pm 21.208$ ), nonetheless, the low nutritional status shown a non-significant difference ( $P>0.05$ ) compared to moderate status ( $39.36\pm 26.6$ ), as shown in figure 3.

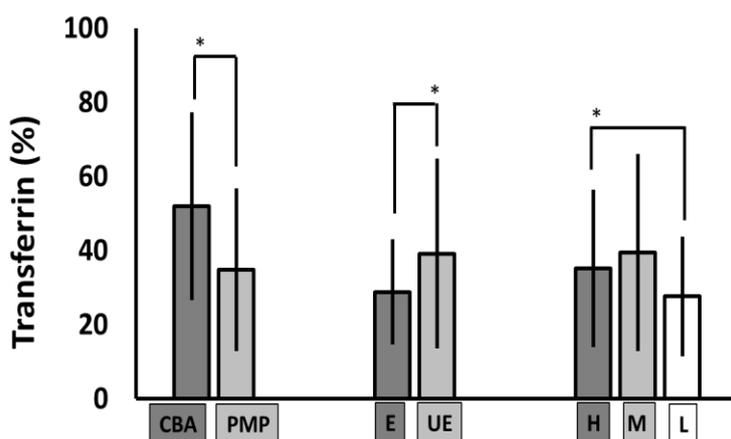
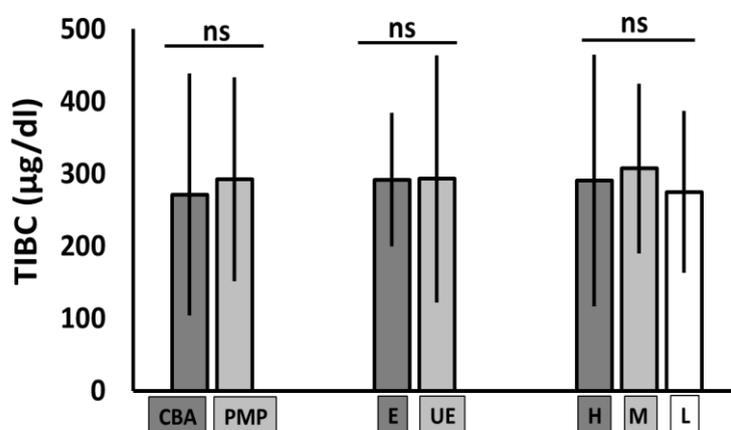


Figure 3. Transferrin significantly reduced with all variables age or employment status or nutritional status. Data expressed as mean±SD. CBA=child-bearing age, PMP= postmenopausal, E=employed, UE=unemployed, H=high nutritional status, M=middle nutritional status, L=low nutritional status.

The result of the total iron-binding capacity test has confirmed a non-significant ( $P>0.05$ ) differences between child-bearing age and postmenopausal women ( $271.31\pm 167.55$  and  $292.42\pm 141.21$ , respectively) and non-significant ( $P>0.05$ ) differences exist between employed and unemployed women ( $291.85\pm 92.66$  and  $292.83\pm 170.67$ , respectively). Moreover, non-significant ( $P>0.05$ ) differences exist based on the nutritional status of women, whether high,

moderate or low (290.57±174.11, 307.3±117.47, or 274.86±112.21, respectively), as shown in figure 4.



**Figure 4.** Iron binding capacity shows no change in association with age or employment status or nutritional status. Data expressed as mean±SD. CBA=child-bearing age, PMP=postmenopausal, E=employed, UE=unemployed, H=high nutritional status, M=middle nutritional status, L=low nutritional status, ns=non-significant.

## DISCUSSION

Derangement of iron metabolism bestowed a great impact on subjects; vitiating overall daily physical and mental activities of the affected patient. Epidemiological studies entrenched a logical more pervasive association of iron imbalances in females compared to males. Physical parameters, such as menstrual cycle, and child-bearing ages could preeminence logical factors in the essence of anemia. Nonetheless, lifestyle and socioeconomic status carry less attention compared to physical parameters(15). The present study demonstrated that nutrition and employment should be considered in the context of anemia diagnosis if any.

Despite being exclusively linked to hemeproteins (e.g. hemoglobin) synthesis and red blood cells formation; however, iron encompasses other roles, namely cytochromes function, which is implicated in carrying oxygen and coupling the electron transport chain of the mitochondria and cellular metabolism, as well as catalases and peroxidases; which are enzymes that participate in oxidation-reduction reactions. These vital functions are increasingly affected when the iron status changes by stress-mediated (employability or workplace stress) or malnutrition, leading to physical and mental problems.

In terms of pathogenesis, periodic bleeding is perhaps the most prevalent cause of iron deficiency and iron-deficiency anemia in women of child-bearing age. Women who have excessive monthly bleeding are more prone. However, our study connoted that transferrin insignificantly reduced in women at child-bearing age compared to postmenopausal women. Nonetheless, other measured parameters (TIBC, Serum Iron, and Hb) were not modulated in either case and under all circumstances; denoting that the deleterious effect is partial and might be of non-clinical significance. The result of this study disagree with a study conducted by Chiheb et al. 2017, on 140 pregnant women and the study concluded that all participant have some sort of anaemia with approximately one-half of them were assigned as iron deficiency anaemia; this differences could be explained in the context of increased demands for nutritional substances in pregnant women(16). An alternative study conducted by Fernandez-Jimenez et al., 2020, confirmed that the young age group at childbearing age show more prevalence of physical and mental anaemia-linked symptoms including pica, cheilitis, restless legs syndrome diffuse hair loss, and ungual alterations, and these symptoms appear to be more prominent in younger than postmenopausal age (17). Despite the aforementioned importance of iron in child-bearing age; the maintenance of normal iron level in postmenopausal age are still important for normal health. A study conducted by Jian et al. 2009, revealed that preservation of normal iron status in postmenopausal age and hormonal changes should be practically considered (18). Moreover, overloaded iron due to postmenopausal age needs careful treatment to avoid toxicity because there are reports about their association with bone diseases in the elderly or postmenopausal age (19).

Absorption maintains cellular and body iron quasi-equilibrium because it's one-way direction i.e. absorbed with no excretion. Absorption 1-2mg is considerably low compared to intracellular concentration, therefore, deficiency is relatively odd. Nevertheless, working hours and workplace stress alongside malnutrition could provoke the ensuing anaemia or mild status of iron deficiency.

Nutrition provides the body with iron absorption enhancers and inhibitors. Our result concluded that low nutritional values are directly linked to the presence of subclinical anaemia. In a study conducted by Root et al, 1999, in different districts of China with various nutritional habits (20). The study sample was women of child-bearing age. The study found that food is linked directly to the incidence of the presence of subclinical anaemia. The study found that the correlation is remarkably positive between iron status and food quality and quantity and some food habits could directly suppress iron absorption from GIT like drinking

tea immediately after food. The study also confirmed that type of food whether of plant origin or animals are directly linked to the serum iron and iron-related parameters. An alternative systematic review involved 49 European studies in 29 countries conducted by Milman in 2019, the study concluded that nutritional value is a considerably important factor for anaemia prevalence in the overall population. According to this research, a substantial proportion of women of childbearing age in Europe had a dietary iron consumption of less than 15 mg/day(2). A poor iron intake may result in a reduced body iron status observed in many European women. There is indeed a clear utmost need for European nations within the European Union for the establishment and operation of shared regulated dietary approaches, as well as the harmonization of nutrition facts tables, as recently proposed by European Food Safety Authority(21). In order to achieve valid international assessments of nutrient habits of macro-and micronutrients, it is also necessary to reach an agreement on the use of the various Dietary Reference Values and to enforce the use of standard statistical methodologies.

## **CONCLUSION**

The lifestyle and routine daily nutritional status of the individual greatly impacted the iron status and iron-linked parameters. Our study concluded that nutritional status affects haemoglobin concentration regardless of age or workplace stress of employability. Despite that iron or its binding capacity; neither show changes based on menstruation nor, it does show changes based on nutritional status. While transferrin level was greatly impacted by nutritional status, age, and employability. We do recommend the administration of iron supplements to these categories who are at high risk for anaemia.

## **Author Contribution**

Conception and design: EHA and ZMA; Acquisition, analysis and interpretation of data: EHA and MKJA; Drafting the article: MKJA and ZMA

**Conflict of interests:** The authors declare no potential conflict of interests.

**Acknowledgement:** The authors are very grateful to the college of Medicine and College of Pharmacy in the University of Mosul for their provided facilities to conduct this study.

## **FUNDING**

No specific funding was received for this study.

## References

1. Anderson GJ, Frazer DM. Current understanding of iron homeostasis. *Am J Clin Nutr.* 2017;106:1559S-1566S.
2. Milman NT. Dietary iron intake in women of reproductive age in Europe: A review of 49 studies from 29 countries in the period 1993-2015. *J Nutr Metab.* 2019;2019.
3. Milman N. Anemia - Still a major health problem in many parts of the world! *Ann Hematol.* 2011;90(4):369–77.
4. Milman N, Pedersen P, Steig T, Byg KE, Graudal N, Fenger K. Clinically overt hereditary hemochromatosis in Denmark 1948-1985: Epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. *Ann Hematol.* 2001;80(12):737–44.
5. Milman N, Taylor CL, Merkel J, Brannon PM. Iron status in pregnant women and women of reproductive age in Europe. *Am J Clin Nutr.* 2017;106(C):1655S-1662S.
6. Shander A, Javidroozi M, Ozawa S, Hare GMT. What is really dangerous: Anaemia or transfusion? *Br J Anaesth.* 2011;107(SUPPL. 1):41–59.
7. Deal JA, Carlson MC, Xue QL, Fried LP, Chaves PHM. Anaemia and 9-year domain-specific cognitive decline in community-dwelling older women: The women's health and aging study II. *J Am Geriatr Soc.* 2009;57(9):1604–11.
8. Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, et al. Iron deficiency anemia and cognitive function in infancy. *Pediatrics.* 2010;126(2).
9. Wilson C, Brothers M. Iron Deficiency in Women and Its Potential Impact on Military Effectiveness. *Nurs Clin North Am (Internet).* 2010;45(2):95–108. Available from: <http://dx.doi.org/10.1016/j.cnur.2010.02.005>
10. Hernández-Martínez C, Canals J, Aranda N, Ribot B, Escribano J, Arija V. Effects of iron deficiency on neonatal behavior at different stages of pregnancy. *Early Hum Dev (Internet).* 2011;87(3):165–9. Available from: <http://dx.doi.org/10.1016/j.earlhumdev.2010.12.006>
11. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J*

- Clin Nutr. 2007;85(4):931–45.
12. Perez EM, Hendricks MK, Beard JL, Murray-Kolb LE, Berg A, Tomlinson M, et al. Mother-infant interactions and infant development are altered by maternal iron deficiency anemia. *J Nutr.* 2005;135(4):850–5.
  13. Murray-Kolb LE, Beard JL. Iron deficiency and child and maternal health. *Am J Clin Nutr.* 2009;89(3):946–50.
  14. Beard JL, Hendricks MK, Perez EM, Murray-kolb LE, Berg A, Vernon-feagans L, et al. and Cognition 1. 2005;(August 2004):267–72.
  15. Mejia CR, Sulca PA, Hernani-Salazar L, Ricaldi-Asto L, Rojas MA, Hernández-Arriaga G, et al. Association of nutritional status and anemia with multi-micronutrient supplementation in young children in Peru. *Electron J Gen Med.* 2019;16(5).
  16. Hadjira C, Kamel AM, Zahia B, El Amin AMY, Arezki B. Iron deficiency anemia and nutritional status among women of childbearing age. *Med J Nutrition Metab.* 2017;10(3):235–42.
  17. Fernandez-Jimenez MC, Moreno G, Wright I, Shih P-C, Vaquero MP, Remacha AF. Iron Deficiency in Menstruating Adult Women: Much More than Anemia. *Women’s Heal Reports.* 2020;1(1):26–35.
  18. Jian J, Pelle E, Huang X. Iron and menopause: Does increased iron affect the health of postmenopausal women? *Antioxidants Redox Signal.* 2009;11(12):2939–43.
  19. Chen B, Li GF, Shen Y, Huang X, Xu YJ. Reducing iron accumulation: A potential approach for the prevention and treatment of postmenopausal osteoporosis. *Exp Ther Med.* 2015;10(1):7–11.
  20. Root MM, Hu J, Stephenson LS, Parker RS, Campbell TC. Iron status of middle-aged women in five counties of rural China. *Eur J Clin Nutr.* 1999;53(3):199–206.
  21. Kearney J. Rationale and methods of the EFCOSUM project. *Eur J Clin Nutr.* 2002;56:S4–7.