Advanced Medical Journal, Vol.10, No.1, P.50-58,2025 https://doi.org/10.56056/amj.2025.317

Serum levels of ferritin and vitamin B12 in a cohort of healthy non-anemic female medical students

Suleen Kanan Bakir* Rawand Polus Shamoon Ranan Kardagh Polus*****

Abstract

Background and objectives: Iron and Vitamin B12 are essential for the development of the nervous system, erythropoiesis, and several metabolic functions. This study aimed to assess serum levels of ferritin and vitamin B12 in a cohort of apparently normal young non-pregnant females.

Methods: Between October 2022 and February 2023, a descriptive cross-sectional study was carried out at Knowledge University in Erbil, Kurdistan, Iraq. 400 young female college students who appeared normal and non-anemic underwent tests for vitamin B12, serum ferritin, and total blood count. Their eating habits and menstrual history were investigated and connected to the biochemical findings.

Results: A statistically significant correlation between low ferritin and B12 levels was found in 48 (12%) of the students. Additionally, among the participated students, 96 (24%) and 114 (28.5%) had low serum levels of vitamin B12 and ferritin, respectively. Between 4 and 120 ng/L, the mean serum ferritin level was 44.46 ng/L. Between 67 and 600 pg/mL, the mean vitamin B12 level was 257.9 pg/mL. The average hemoglobin, corpuscular volume, and hemoglobin of the students who were enrolled were 12.88 g/dL, 85.98 fL, and 27.43 pg, respectively.

Conclusion: Low vitamin B12 levels were mostly found in vegetarians and people on non-ketogenic diets, while low serum ferritin levels were much more common in people with abnormal menstruation. Iron and vitamin B12 deficiencies are frequent in otherwise healthy girls with normal hemoglobin and red blood cell indices.

Key words: Anaemia, Ferritin, Vitamin B12, Young female.

* M.B.Ch.B. Candidate of KHCMS of Hematology, Ministry of Health, Erbil, Kurdistan Region, Iraq. Email: suleen.kanan@gmail.com, Corresponding author
** M.B.Ch.B, MSc, PhD, Department of Pathology, College of Medicine, Hawler Medical University, Erbil Iraq & Department of Laboratory Medical Sciences, College of Health Sciences, Catholic University, Erbil, Iraq. Email: rawand.shamoon@hmu.edu.krd
***M.B.Ch.B, MSc, PhD, Department of Basic Sciences, College of Medicine, Hawler Medical University, Erbil, Iraq, Email: ranan.kardagh@hmu.edu.krd

Introduction

Deficiencies of micronutrients form an important global health issue as they affect physical and mental development. Iron and vitamin B12 are considered as two main nutrients that are involved directly in erythropoiesis. Iron is vital for many metabolic processes, including oxygen transport, DNA synthesis, and muscle metabolism.[1] Iron deficiency is a significant human health concern; it is the cause of the most prevalent nutritional deficiency worldwide, affecting about one-third of women and 43% of children worldwide.[2] Even in absence of anemia, iron deficiency seems to be associated with clinical signs and symptoms as easy fatigability and impaired of physical performance.[3] Inadequate dietary intake of iron or failure of its absorption are not uncommon reasons of iron deficiency. Menstruation is the most common cause of blood loss and it is the primary reason that iron deficiency is more common in women. Children and adolescents and pregnant women require more iron and are more prone for iron deficiency.[3] Ferritin is the primary iron-storage protein and is critical to iron homeostasis. The level of ferritin in the serum therefore reflects the amount of iron stored in the body. Ferritin is an acute-phase reactant; in the absence of inflammation, serum ferritin level correlates positively with the size of the total body iron stores.[4] Hence, when iron deficiency is expected in the presence of a chronic inflammatory process, transferrin saturation (TSAT) is a better indicator for detection of iron deficiency.[5] Vitamin B12 is considered as an important micronutrient that has various important roles in the body such as macronutrient metabolism, the formation of red blood cells and DNA.[6] This substance can only be gained from animal derived foods, as human body cannot synthesize it; for this reason, vegetarians are very susceptible to vitamin B12 deficiency.[7] Lack of vitamin B12 causes serious health problems, such as anemia, severe neurological dysfunction, and metabolic illnesses, as well as a rise in the biochemical risk markers for disease.[8] According to Hinkel,[9] the liver holds the majority of the body's usual total vitamin B12 reserves, which are 3-5 mg. It is first coupled to an intrinsic factor made by gastric parietal cells in the duodenum and jejunum, and then it is absorbed in the terminal ileum.[10] As a result, when vitamin B12 levels in the diet are low or absent, stores may persist for up to 10 years before clinical appearance of vitamin B12 deficiency. In most cases, malabsorption-related conditions such autoimmune gastritis, inflammatory bowel diseases, surgical gastrectomy, and ileal resection are the causes of vitamin B12 insufficiency.[6,11] The frequency of vitamin B12 deficiency among those who follow vegetarian diets was studied by Pawlak.[12] This research indicated a comparatively high prevalence of deficiencies among vegetarians. The British Society for Hematology updated its recommendations for vitamin B12 replacement recently.[13] Ketogenic (keto) diets have grown in popularity as a strategy to lose weight by limiting carbohydrates and replacing them with a lot of healthy fats that the human body uses as fuel.[14] According to previous research, Vitamin B12 insufficiency has been associated with aberrant brain development and behavior. Whether vitamin B12 insufficiency is a risk factor for fetus abnormalities, particularly neural tube defects (NTDs), is a topic of continuous discussion.[15] Recent studies have revealed that folic acid fortification alone may not be as efficient at lowering NTDs as folic acid in combination with vitamin B12.[16,17] Within a sample of young female medical students who appeared to be in good health, we intended to particularly assess the levels of ferritin and vitamin B12 in the current study. The objective of this study was to investigate

https://doi.org/10.56056/amj.2025.317

the relationship between the group's dietary practices and menstrual bleeding and the proportion of those two micronutrient deficits.

Materials and Methods

In this descriptive cross-sectional study; we conveniently selected a group of apparently healthy non-anemic young female medical students whose age ranged between 18 to 25 years. The participants were Knowledge University students from Erbil City, Kurdistan, Iraq. Between October 2022 and February 2023, volunteers were selected, and samples were collected. The enrolled students agreed to participate in the study and filed their written consent. The study was approved by the Research Ethical Committee of Kurdistan Higher Council of Medical Specialties (KHCMS). Female students with history of acute or chronic illnesses, taking drug or supplements, pregnant or breastfeeding were not included. Moreover, data of many others who have had Hemoglobin (Hb) level below 11.5g/dL were excluded. According to the ethic document no. 1391 dated 14/08/2022 data of 400 female students who fulfilled our inclusion criteria was finally analyzed. The menstrual status was assessed by asking the students their menstrual cycle (polymenorrhea, hypermenorrhea, normal) and nutritional habits was evaluated through asking about vegetarian, non-vegetarian and dietary habit including who's in diet or not on diet. Further, students that are on diet are divided to kieto and non-keto. Polymenorrhea was defined as menstruation occurring more frequently than once within less than 21 days. Hypermenorrhea was defined as prolonged menstrual bleeding at regular intervals (more than 8 days).[18] The nutritional habit of the enrolled students was assessed and the enrollees were categorized to vegetarian and non-vegetarian. Those who were on dietary system were grouped according to the type of dietary regimen into keto diet and non-keto diet. Six ml of venous blood was withdrawn from the enrolled students and was collected in K3-EDTA(3ml) and gel separator tubes(3ml). Complete blood count (CBC) was performed using Sysmex® XP hematology analyzer. Serum levels of vitamin B12 and ferritin were measured using commercially available kits on Cobas® 6000, Roche, Hitachi, Germany. Serum ferritin levels <12 µg/L and serum B12 <200 pg/mL were considered as deficiency in students with normal CBC parameters.17 Results of serum ferritin and B12 levels were analyzed and correlated with the menstrual and nutritional status. Further, in this study the statistical methods that have been used are ANOVA test, independent sample t-test, and Chi-square test.

Results

The mean Hb level of the participants was 12.88 g/dL (±0.80) ranged from 11.5 to 15.0 g/dL. The mean MCV and MCH were 85.98 fL (±4.41) and 27.43 pg (±1.65) ranged from 79 to 98 fL and 25 to 31 pg respectively. The mean serum ferritin was 44.46 µg/L (±30.42) ranged from 4 to 120 µg/L. The mean serum vitamin B12 level was 257.9 pg/mL (±106.40) ranged from 67 to 600 pg/mL shown in Table (1). Within the studied group, 96(24 %) had low serum ferritin levels and 114 (28.5%) had low vitamin B12 levels.

Table (1): mean and standard deviation of hematological parameters

	Mean	Standard Deviation	Minimum	Maximum
HB	12.88	0.80	11.50	15.00
MCV	85.98	4.41	79.00	98.00
MCH	27.43	1.65	25.00	31.00
FERRITIN	44.46	30.42	4.00	120.00
B12	257.91	106.40	67.00	600.00

https://doi.org/10.56056/amj.2025.317

Polymenorrhea and hypermenorrhea were encountered in 40 (10%) and 48 (12%) of the enrolled students respectively. The Hb and red cell parameters together with serum ferritin and B12 levels of students with abnormal menstrual bleeding are illustrated in Table (2). As can be seen in the table, the value of standard deviation is very small which means the homogeneity of data is very strong. Thus, any small change occurs will be significant. The Hb and serum ferritin level were significantly lower among the students with excessive bleeding during menstruation comparing to those with normal menstruation.

Table (2): Red cell indices and serum ferritin and B12 levels of students with abnormal menstruation

		N	Mean	Std. Deviation	p-value
Hb (g/dL)	Polymenorrhagia	40	12.59	0.81	0.043
	Hypermenorrhea	48	12.64	0.56	
	Normal	312	12.96	0.82	
	Total	400	12.88	0.80	
MCV (fL)	Polymenorrhagia	40	84.95	4.04	0.248
	Hypermenorrhea	48	85.04	4.69	
	Normal	312	86.26	4.37	
	Total	400	85.98	4.40	
MCH (pg)	Polymenorrhagia	40	27.20	1.56	0.337
	Hypermenorrhea	48	27.04	1.47	
	Normal	312	27.52	1.67	
	Total	400	27.43	1.64	
Ferritin (µg/L)	Polymenorrhagia	40	30.90	29.85	0.001
	Hypermenorrhea	48	15.04	15.38	
	Normal	312	50.72	28.99	
	Total	400	44.46	30.38	
B12 (pg/mL)	Polymenorrhagia	40	240.85	136.91	0.752
	Hypermenorrhea	48	258.46	114.79	
	Normal	312	260.01	100.52	
	Total	400	257.91	106.27	

*ANOVA test is used.

https://doi.org/10.56056/amj.2025.317

About 15 (3.75%) of the students in the study group were vegetarians, and 84 (21%) had dietary restrictions. 42 people, or 10.5% of those on a diet, were following a ketogenic diet. The Hb and red cell parameters, as well as the serum ferritin and B12 levels, are shown in Table (3) for students following a diet plan. The criteria for vegetarian and non-vegetarian students are shown in Table (4). Both vegetarian students and those on a diet plan had noticeably decreased serum B12 levels. Hb was lower in vegetarians, but there was not a significant distinction in the other measures.

Table (3): Red cell indices and serum ferritin and B12 levels of students on dietary regimen

		N	Mean	Std. Deviation	p-value
Hb (g/dL)	Keto diet	42	13.01	0.87	0.155
	Non-keto diet	42	12.68	0.64	
	Not on diet	316	12.89	0.81	
	Total	400	12.88	0.80	
MCV (fL)	Keto diet	42	85.71	4.19	0.675
	Non-keto diet	42	85.38	4.36	
	Not on diet	316	86.09	4.44	
	Total	400	85.98	4.40	
MCH (pg)	Keto diet	42	27.33	1.69	0.246
	Non-keto diet	42	27.19	1.49	
	Not on diet	316	27.47	1.66	
	Total	400	27.43	1.64	
Ferritin (µg/L)	Keto diet	42	51.19	32.84	0.342
	Non-keto diet	42	35.43	31.50	
	Not on diet	316	44.76	29.71	
	Total	400	44.46	30.38	
B12 (pg/mL)	Keto diet	42	247.52	98.66	0.001
	Non-keto diet	42	185.38	105.01	
	Not on diet	316	268.92	103.75	
	Total	400	257.91	106.27	

*ANOVA test is used

https://doi.org/10.56056/amj.2025.317

Table (4): Red cell indices and serum ferritin and B12 levels of vegetarian and non-vegetarian students

Vegetarian		N	Mean	Std. Deviation	p-value
Hb (g/dL)	Non-Vegetarian	385	12.90	0.80	0.004
	Vegetarian	15	12.28	0.67	
MCV (fL)	Non-Vegetarian	385	86.02	4.35	0.143
	Vegetarian	15	85.00	5.64	
MCH (pg)	Non-Vegetarian	385	27.45	1.64	0.312
	Vegetarian	15	27.00	1.81	
Ferritin (µg/L)	Non-Vegetarian	385	44.94	30.38	0.146
	Vegetarian	15	32.13	28.90	
B12 (pg/mL)	Non-Vegetarian	385	261.58	105.46	<0.001
	Vegetarian	15	163.67	82.99	

Within 400 students we found 48 (12%) students having both ferritin and vitamin B12 levels low, while 48(12%) having only low ferritin and 66(16.5%) having only vitamin B12 deficiency with the other 238(59.5%) having normal ferritin and vitamin B12. The association between low levels of serum ferritin and vitamin B12 was found to be significant ($p<0.001$) as illustrated in Table (5).

Table (5): Relation between serum ferritin and vitamin B12

Serum B12						
		Low		Normal		p*
		Count	%	Count	%	
Serum Ferritin	Low	48	12%	48	12.0%	p<0.001**
	Normal	66	16.5%	238	59.5%	

*Chi-square test is used

Table (6) compares the red cell indices between students with low versus normal serum ferritin and B12 levels. The mean Hb, MCV and MCH were significantly lower among students with low ferritin level compared to those with normal ferritin. The mean Hb was significantly lower among students with low serum B12 level; however, the means of MCV and MCH did not significantly differ between those with low and normal B12 level. When excluding the 48 students with combined low ferritin and B12 levels; the means of Hb, MCV and MCH remained significantly lower in those with low ferritin level, but did not show any significant difference between students with low and normal B12 level.

https://doi.org/10.56056/amj.2025.317

Table (6): Red cell indices in relation to ferritin and vitamin B12 levels

	Total	No. (%)	Mean (SD)		
			Hb (g/dL)	MCV (fL)	MCH (pg)
Ferritin <12 µg/L	400	96 (24)	12.38 (0.57)	83.50 (3.77)	26.56 (1.33)
Ferritin >12 µg/L		304 (76)	13.04 (0.80)	86.76 (4.30)	27.70 (1.64)
p-value			<0.001	<0.001	<0.001
Ferritin <12 µg/L	352*	48 (13.6)	12.49 (0.57)	83.63 (3.60)	26.54 (1.27)
Ferritin >12 µg/L		304 (86.4)	13.04 (0.80)	86.76 (4.30)	27.70 (1.64)
p-value			<0.001	<0.001	<0.001
B12 <200 pg/mL	400	114 (28.5)	12.61 (0.78)	85.47 (4.77)	27.26 (1.64)
B1>200 pg/mL		286 (71.5)	12.99 (0.79)	86.18 (4.24)	27.50 (1.65)
p-value			<0.001	0.073	0.100
B12 <200 pg/mL	352*	66 (18.8)	12.86 (0.83)	87.00 (4.75)	27.76 (1.63)
B12 >200 pg/mL		286 (81.2)	12.99 (0.79)	86.18 (4.24)	27.50 (1.65)
p-value			0.116	0.084	0.123

* Students with combined low ferritin and B12 (n=48) were not included and independent sample t-test is used

Discussion

Nutritional deficiencies remain a significant topic among young female group worldwide.This is attributed to many diverse causes, of which bleeding and dietary habits are among the top. Iron deficiency without anemia represent a diagnostic challenge as there are no well-defined criteria for diagnosis of this condition, also because it may go unrecognized for long period.[19] Ferritin is an indicator of iron stores and is the most sensitive and specific biomarker for assessing iron deficiency in otherwise apparently healthy individuals.[3] The suspicion of iron deficiency without anemia should arise if a patient with normal CBC presents symptoms of iron deficiency anemia primarily together with low ferritin concentration and especially when the medical history supports iron deficiency.[19] A total of 400 young students who appeared to be healthy and had normal hemograms made up the samples for this study. The study's objective is to determine how frequently young female students who are otherwise normal have low ferritin and/or low B12 levels. We believe that our sample is quite representative and precise for this screening as we excluded supplement taking individuals and pregnant ladies which are the main confounding factors. Interestingly, considerable proportions of the enrollees found to have low serum ferritin (96, 24%) and/or low serum vitamin B12 (114, 28.5%). It is noteworthy mention that the sample size is not computed because of large sample size since the data sample of 400 students from different families in the population were collected. It is a well-known fact that the sample size will be computed for small dataset of a population. In our study, 22% of the enrolled students had excessive or

https://doi.org/10.56056/amj.2025.317

irregular menstruation; 12% had hypermenorrhea and 10% had polymenorrhea. Their mean ferritin level was significantly lower; however, their Hb and red cell parameters did not vary significantly. Comparable rates of hypermenorrhea and polymenorrhea were recorded at 12.94% and 9.94% respectively in Iran.[18] According to a study done in Turkey over 466 females who were screened before marriage for ferritin and B12, the rates of low ferritin and B12 were 46.1% and 21.6% respectively; though, 25% of the included women were anemic.[20] Our study differs from this one in that we excluded anemic females from our sample, the rate of low serum ferritin level was 17.3%.[21] There are regional variations in vitamin B12 levels. Studies have revealed that certain populations have higher rates of vitamin B12 deficiency. In Jordan, 30.1% of women over the age of 19 years who participated in one survey that included different age groups and genders had vitamin B12 deficiencies.[22] In our study the rate of low serum B12 was a little bit lower; a possible reason for the difference is that we excluded anemic females from our survey. Deficiency of vitamin B12 is mostly related to insufficient intake of meat and animal proteins because of peculiar nutritional habits or poverty. The current study ensures this relation as majority of the enrolled students who were on non-keto diet and those who were vegetarian had low serum B12 level. After controlling the confounding variables, we determined that dietary habits were the main risk factor for vitamin B12 deficiency. In few other studies, daily sedentary behavior, low income, and age were found to be the key risks.[23] Many studies have explored the relationship between economic status and vitamin B12 deficiency risk. They concluded that low-income individuals may have limited access to a varied and nutritious diet, which could contribute to deficiency in vitamin B12.[18] A considerable number of the enrolled students (48, 12%) found to have low ferritin and B12 levels at the same time. Majority of the latter group had bleeding and were on diet or were vegetarians. The simultaneous low level of ferritin and B12 is mostly attributed to the cumulative effect of the bleeding and nutritional deficiencies caused by dietary restrictions. All enrolled students had normal red cell parameters; however, the Hb concentration varied significantly between the ferritin and B12 deficient and the non-deficient groups. The parameters of the red cells did not vary significantly between the B12 deficient and B12 non-deficient groups even after exclusion of students with combined low B12 and ferritin.

Conclusion

Low levels of serum ferritin and B12 were detected in a significant proportion of apparently normal young female students in spite of having normal Hb and normal red cell indices. Respectively, low ferritin and B12 levels were significantly related to bleeding and dietary habits.

Conflicts of interest

There were no conflicts of interest.

References

1. Netz DJ, Mascarenhas J, Stehling O, Pierik AJ, Lill R. Maturation of cytosolic and nuclear iron – sulfur proteins. Trends Cell Biol. 2014;24(5):30312.
2. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. Lancet Glob Health. 2013;1(1): 16–25.
3.World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and

https://doi.org/10.56056/amj.2025.317

populations. Geneva: WHO, 2020. <https://www.who.int/publications-detail-redirect/9789240000124>
4. Koperdanova M, Cullis J O. Interpreting raised serum ferritin levels. BMJ 2015; 22(3): 351-59.
5. Al-Naseem A, Sallam A, Choudhury S, and Thachil J. Iron deficiency without anaemia: a diagnosis that matters. Clin Med (Lond). 2021;21(2):107-13.
6. Green R, Mitra A. Megaloblastic Anemias: Nutritional and Other Causes. Med Clin North Am. 2017;101(2):297-317.
7. Ray J, Meier C, Vermeulen M, Boss S, Wyatt P, Cole DEC. Association of Neural Tube Defects and Folic Acid Food Fortification in Canada. The Lancet, 2002. 360(9350):2047-8.
8. Wilhelm S, Rjater R, Kale-Pradhan P. Perils and Pitfalls of Long-term Effects of Proton Pump Inhibitors. Expert Rev. Clin. Pharmacol.2013, 6(4):443-51.
9. Hinkel J, Schmitt J, Wurm M, Rosenbaum-Fabian S, Schwab KO, et.al. Elevated Plasma Vitamin B12 in Patients with Hepatic Glycogen Storage Diseases. J Clin Med. 2020; 9(8):2326-36.
10. Hunt A, Harrington D and Robinson S. Vitamin B12 deficiency. BMJ. 2014; 10(3): 349-59.
11. Stabler S. Vitamin B12 deficiency. N Engl J Med, 2013; 368:149-60.
12. Pawlak R, Lester SE and Babatunde T. The Prevalence of Cobalamin Ceficiency Among Vegetarians Assessed by Serum Vitamin B12: A Review of Literature. Eur J. Clin Nutr. 2014;68(5):541-48.
13. Azzini E, Raguzzini A and Polito A. A Brief Review on Vitamin B12 Deficiency Looking at Some Case Study Reports in Adults. Int J Mol Sci., 2021;22(18):9694-710.
14. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and Appetite-mediating Nutrients and Hormones after Weight Loss. Eur J Clin Nutr. 2013;67(7):759-64.
15. Molloy AM. Should vitamin B12 status be considered in assessing risk of neural tube defects? Ann N Y Acad Sci. 2018:1414(1):109-125.
16. Allen LH, Miller JW, de Groot L, Rosenberg IH, Smith AD, Refsum H, et al. Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review. J Nutr. 2018 1;148(4):1995S-2027S.
17. Karakaş NM. The Prevalence of Low Serum Levels of Vitamin D, Vitamin B12, Folate and Ferritin in Adolescents: Single Center Experience. Sci Prog. 2021;104(2). 368-99.
18. Samani RO, Hashiani AA, Razavi M, Vesali S, Rezaeinejad M, Maroufizadeh S., et al. The Prevalence of Menstrual Disorders in Iran: A Systematic Review and Meta-Analysis. Int J Reprod Biomed. 2018;16(11):665-78.
19. Soppi E. Iron deficiency without anemia–common, important, neglected. Clin. Case Rep. Rev, 2019; 5:1–7.
20. Karabulut A, Güler ÖT, Karahan HT, Özkan S, Koyuncu H, Demirciler I. Premarital screening of 466 Mediterranean women for serum ferritin, vitamin B12, and folate concentrations. Turk J Med Sci. 2015;45(2):358-63.
21. Kocaoz S, Cirpan R, Degirmencioglu AZ. The Prevalence and Impacts Heavy Menstrual Bleeding on Anemia, Fatigue and Quality of Life in Women of Reproductive Age. Pak J Med Sci. 2019;35(2):365-70.
22. El-Khateeb M, Khader Y, Batieha A, Jaddou H, Hyassat D, et.al. Vitamin B12 Deficiency in Jordan: A Population-based Study. Ann Nutr Metab. 2014;64(2):101-5.
23. Al-Musharaf S, McTernan PG, Hussain SD, Aleisa KA, Alnaami AM, et.al. Prevalence and Indicators of Vitamin B12 Insufficiency among Young Women of Childbearing Age. Int J Environ Res Public Health. 2020;18(1):1-17.

https://doi.org/10.56056/amj.2025.317