RESEARCH ARTICLE

Examination and analyzing the levels of related micronutrients and anemia in pregnant women

Asma'a Hassan Mohamed[1], Yasir Haider Al-Mawlah[2,*], Hadi Sajid Abdulabbas[3]

[1]Department of Medical Laboratory Techniques, Al-Mustaqbal University College, Babylon 51101, Iraq. [2]DNA Research Centre, University of Babylon, Babylon, Hillah 51001, Iraq. [3]Continuous Education Department, Faculty of Dentistry, University of Al-Ameed, Karbala 56001, Iraq.

Dietary variety and quantity are critical for pregnant women, especially anemic pregnant women. The micronutrients such as vitamins A, D, E, folate, B12, B6, and C, iron, zinc, iodine, copper, and selenium have received the most attentions in pregnancy. Severe iron deficiency anemia during pregnancy increases the risk of premature birth. The aim of this study was to investigate the relationship between micronutrients dietary and variety and anemic pregnant women. A total of 250 pregnant women in Babylon province from February 2021 to March 2022 were selected as the survey objects. Fasting venous blood samples were taken to detect hemoglobin and anemia-related micronutrients. The pregnancy outcomes and physical indicators of newborns were followed up. Among 250 pregnant women, the incidences of deficiencies of vitamin A, borderline vitamin A, iron, and vitamin B12 were 12.0% (30/250), 43.6% (109/250), 5.6% (14/250), and 0.4% (1/250), respectively. The incidence of pregnancy anemia was 29.9% (61/204). The plasma vitamin A and iron concentrations of pregnant women with anemia group in the first trimester were significantly lower than that in the non-anemia group ($P < 0.05$). The birth weight of the newborns in the anemia group in the third trimester was significantly lower than that in the non-anemia group ($P < 0.05$). The incidence of pregnancy anemia and vitamin A and iron deficiency during pregnancy is relatively high in Babylon province. It is necessary to strengthen nutrition and diet guidance during pregnancy and timely treatment to ensure the health of mothers and babies.

Keywords: anemia; vitamin A; vitamin B12; iron; pregnancy.

[*]**Corresponding authors:** Yasir Haider Al-Mawlah, DNA Research Centre, University of Babylon, Babylon, Hillah 51001, Iraq. Phone: +964 770 571 3626. Email: Yasser.almawla@uobabylon.edu.iq.

Introduction

Anemia is one of the most common clinical symptoms during pregnancy, and domestic statistics show that the incidence of pregnant anemia is more than 30% [1]. Anemia during pregnancy is not just caused by iron deficiency. Other micronutrients such as folic acid, vitamin B12, and vitamin A also play the roles in the development of anemia in pregnant women. In other words, anemia is the results of the deficiencies of several nutrients other than iron alone. The collectively caused deficiencies in the basic components or compounds that the body needs will also, in turn, reflect in the amount of nutrients that reach the fetus in pregnant women [1–3]. Micronutrient deficiencies in Iraq are widespread, especially in poorer areas, where it is associated to poverty and malnutrition, particularly among women and girls due to the result of frequent blood loss during menstruation. The fetoplacental unit's requirement for iron increases dramatically during pregnancy, as does the need to compensate for blood loss after birth. It is also commonly caused by a decrease in the intake or

Table 1. The normal values of hemoglobin and micronutrients in non-pregnant and pregnant woman.

Test items	Normal value (non-pregnant female)	Normal value (pregnant female)	Deficiency value
Hemoglobin Hb (g/dl)	12.0 - 15.0	11.5 - 16.0	< 10.0
Folic acid (ng/mL)	2.0 – 20.0	2.7 - 17.0	< 2.0
Vitamin B12 (pg/mL)	160 - 950	279 - 966	< 203
Vitamin A (μmol/L)	0.52 - 2.09	0.7 – 8.4	< 0.70
Iron (mmol/L)	10.5 – 23.0	7.2 – 20.0	< 7.1

consumption of iron-rich foods such as red meat, green leafy vegetables, and iron-fortified foods such as cereals and bread. Most studies domestically and abroad focused on the analysis of the correlation between one or two micronutrients and pregnancy anemia [4–8]. There are few investigative studies on the status of multiple anemia-related micronutrients in pregnant women. In this study, the plasma concentrations of folate, vitamin B12, vitamin A, iron, and other micronutrients were examined in 250 pregnant women in Al-Hilla city, Babylon province, Iraq to investigate the relationship between pregnancy anemia and micronutrient deficiencies, and their effects on neonatal physical indicators. The results of this study could provide the references for pregnancy health care and protection of maternal and child health [9].

Materials and methods

Patient selection

This research project was approved by the Research Ethics Committee of the University of Babylon (Babylon, Hillah, Iraq) (Approval No. DSM-55671). Total of 250 pregnant women aged from 18 to 35 years old in the first trimester (within 12 weeks of pregnancy) who received antenatal care from February 2021 to March 2022 in Marjan Teaching Hospital (Hilla city, Babylon Province, Iraq) with no other systemic diseases were voluntary included in this study. Among them, 204 patients were followed up to the postpartum period. All participants signed the informed consent forms. A health status questionnaire including general conditions, previous menstruation, pregnancy and childbirth, previous illnesses, and folic acid and other nutrient supplements taking during and before pregnancy was obtained from all participants. In addition, the pregnancy and pregnancy outcome questionnaires were also collected from postpartum pregnant women, which included delivery mode, pregnancy complications, and neonatal growth and development indicators.

Blood sample collection and analysis

About 5 mL of whole blood was collected from venous of the patients. The blood samples were drawn by using an ethylenediaminetetraacetic acid dipotassium (EDTA) anticoagulated vacuum blood collection tube (K.S Medical, Hangzhou, Zhejiang, China). The plasma was then separated and stored at -60°C for future tests. The plasma concentrations of hemoglobin (Hb), plasma folic acid, erythrocyte folic acid, plasma vitamin B12, plasma vitamin A, iron, and other indicators were measured. The hemoglobin concentration was also checked in the second trimester (13 - 27 weeks) and the third trimester (≥ 28 weeks). In brief, 1 mL of blood sample was used for on-site hemoglobin detection by using ABX-MICROS automatic blood cell analyzer (Horiba Medical, Kyoto, Japan). The concentrations of folic acid and vitamin B12 were determined by using Folic Acid and Vitamin B12 ELISA Kits (Attogene, Austin, TX, USA) and Beckman Coulter Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA). Vitamin A and iron concentrations were detected by using Waters 2487 Dual Wavelength Absorbance Detector (Waters, Milford, MA, USA) and atomic absorption spectrometry (Perkin Elmer Life Sciences, Waltham, MA, USA) following the manufacturers' instructions for relevant operations [10, 11]. The normal values of micronutrients were listed in Table 1.

Table 2. The blood plasma micronutrient levels in the first trimester.

Micronutrient	Mean ± SD (n = 250)
Plasma vitamin A (µmol /L)	1.09 ± 0.31
Plasma iron (mmol/L)	18.80 ± 6.84
Plasma folic acid (ng/mL)	14.73 ± 5.96
Red blood cell folic acid (ng/mL)	778.27 ± 312.28
Plasma vitamin B12 (pg/mL)	799.86 ± 341.19

Table 3. Micronutrient levels in pregnant women with and without anemia.

Micronutrients	Anemic group (n = 18)	Non-anemic group (n = 186)	*P* value
Plasma folic acid (ng/L)	13. 53 ± 4. 78	14. 15 ± 5. 83	0. 491
Red blood cell folic acid (ng/L)	749.61 ± 223.61	758.82 ± 311.41	0.712
Vitamin B12 (pg/L)	761.128 ±321.75	768.81 ± 330.77	0.901
Vitamin A (umol/L)	0. 97 ± 0. 15	2. 02 ± 0. 27	0.015*
Iron (mmol/L)	13. 49 ± 5. 01	18. 74 ± 7. 06	0.021*

Note: *significant difference ($P < 0.05$).

Statistical analysis

The statistical analysis was performed by using SPSS software (version 20.0) (IBM, Armonk, New York, USA) [12]. Kolmogorow-Smirnov method was used for normality test. The data were expressed as mean ± SD. The t-test was used for comparison of significancy with two-sided tests, and $P < 0.05$ was the indication of significant statistical difference.

Results

Plasma micronutrient levels

Among 250 pregnant women, the incidences of vitamin A deficiency, borderline vitamin A deficiency, iron deficiency and vitamin B12 deficiency were 12.0% (30/250), 43.6% (109/250), 5.6% (14/250), and 0.4% (1/204), respectively. The plasma folic acid and erythrocyte folic acid levels of all subjects were within the normal range (Table 2).

The incidence of anemia in pregnancy

Among 204 pregnant women who were followed up to the postpartum period, the incidence of pregnancy anemia was 29.9% (61/204). The incidence of anemia in the first trimester was 8.8% (18/204), while the incidence of anemia in the second trimester was 13.2% (27/204), of which 13 new cases of anemia occurred in the second trimester. The incidence of anemia in the third trimester was 16.1 % (33/204), including 22 cases of new anemia in the third trimester. With the increase of gestational weeks, the incidence of anemia in pregnancy gradually increased. The incidence of anemia in the second and third trimesters was higher than that in the first trimester.

The relationship between anemia in early pregnancy and micronutrient levels

According to the occurrence of anemia in the first trimester, 204 pregnant women were divided into two groups for comparison of micronutrient levels. Statistical analysis showed that plasma vitamin A and iron concentrations in the anemia group were significantly lower than that in the non-anemia group ($P < 0.05$). There was no significant difference in the mass concentrations of plasma folic acid and erythrocyte folic acid between the two groups ($P > 0.05$) (Table 3).

Effects of anemia in different pregnancy stages on neonates

Statistical analysis results showed that the birth weight and the length of infants from the anemia group of the third trimester were significantly

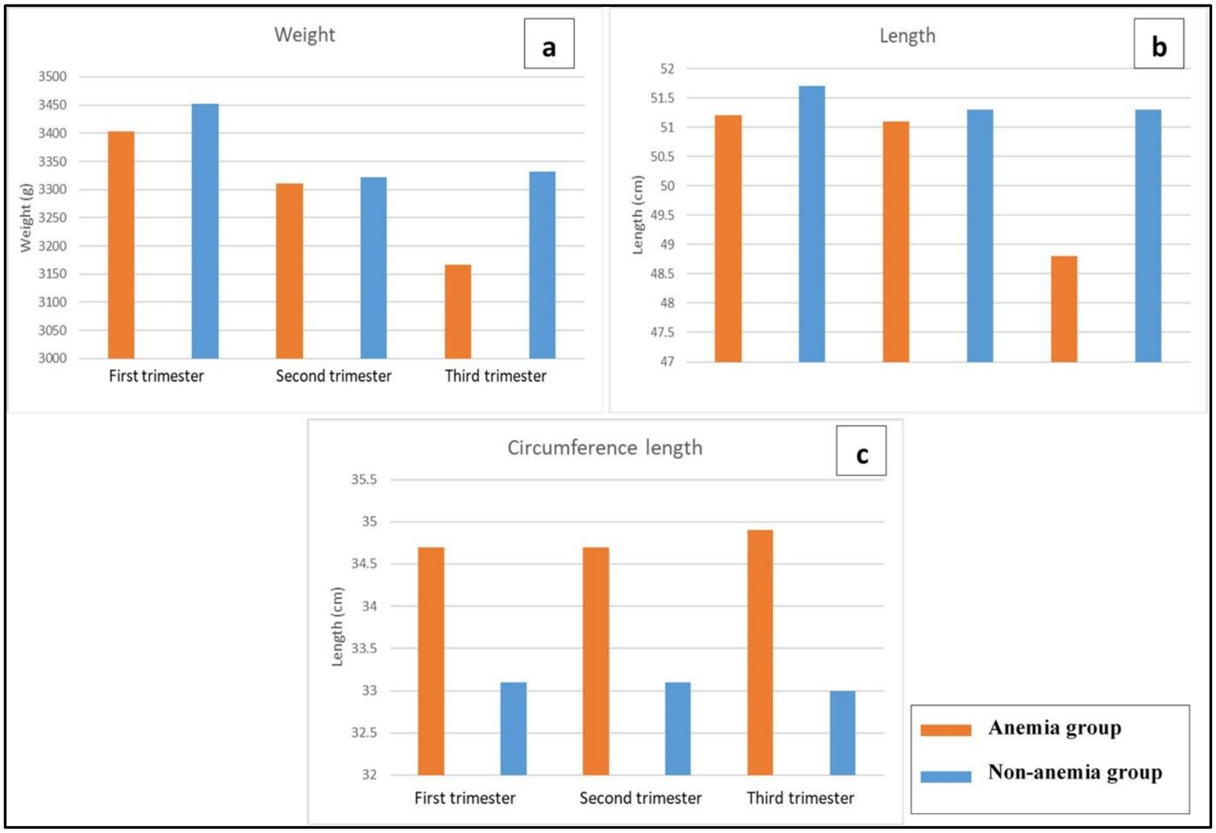

Figure 1. The effects of anemia in pregnant women at different stages of pregnancy on neonates' weight (**a**), length (**b**), and head circumference length (**c**).

Table 4. Comparison of physical indicators of newborns.

Birth index	First trimester		Second trimester		Third trimester	
	Anemia group (n = 18)	Non-anemia group (n = 186)	Anemia group (n = 27)	Non-anemia group (n = 177)	Anemia group (n = 33)	Non-anemia group (n = 171)
Weight (g)	3,403.3 ± 427.5	3,451.6 ± 417.1	3,311.2 ± 405.3	3,322.0 ± 371.27	3,166.6 ± 487.9*	3,331.7 ± 366.2
Length (cm)	51.2 ± 1.7	51.7 ± 1.3	51.1 ± 1.5	51.3 ± 1.2	48.8 ± 1.0**	51.3 ± 1.2
Head circumference (cm)	34.7 ± 1. 0	33.1 ± 0.9	34.7 ± 0.5	33.1 ± 0.9	34.9 ± 0.4	33.0 ± 0.8

Note: *significant difference ($P < 0.05$). **very significant difference ($P < 0.01$).

smaller than that in the non-anemia group in the third trimester with the $P < 0.05$ and $P < 0.01$, respectively (Figures 1a and 1b), while the birth head circumference measurements in non-anemia group were less than that in anemia group. However, there was no significant difference in head circumference measurements between two groups ($P > 0.05$) (Table 4) (Figure 1c).

Discussion

Due to physiological changes, the blood volume of pregnant women will gradually increase with physiological needs, but the increase in the number of red blood cells is slower than that of plasma, resulting in hemodilution and anemia [13–16]. Pregnancy anemia is a high-risk pregnancy, and as the degree of anemia increases, it often leads to abnormal pregnancy and childbirth, affecting the health of mothers and babies. The results of this study showed that the incidence of pregnancy anemia was 29.9%. The study found that the prevalence of anemia in pregnancy was 8.8%, 13.2%, and 16.1% in the first, second, and third trimesters, respectively, showing an increasing trend. The number of new anemia cases in the third trimester was higher than that in the second trimester. This is mainly due to the accelerated growth and development of the fetus in the second and later stages of pregnancy and the increase of blood volume. It is caused by insufficient supply, malabsorption, and loss of protein and micronutrients in the diet. The follow-up studies found that the birth weight of newborns delivered by pregnant women with anemia in the third trimester was significantly lower than that in the non-anemia group. Further analysis also found that about 26.3% of pregnant women with anemia in the first trimester were no longer accompanied by anemia in the second trimester. About 48.6% of the anemia was able to return to normal in the third trimester through routine interventions [14, 17]. In addition, the study found that there was no statistically significant difference in the birth weight of the neonates delivered by pregnant women with anemia in the first and second trimesters comparing to that of non-anemia group, because, if anemia was found in the mid-term, the outpatient physician would give corresponding health education and interventions. Therefore, the preventive measures of early detection, early diagnosis, and early treatment (“three early”) are particularly important in antenatal care and play an important role in promoting maternal and infant health.

Due to the growth and development of the fetus and the needs of the mother herself, the needs of various nutrients in pregnant women increase, and it is often difficult to meet the needs through the ordinary diet. So pregnancy is prone to a variety of nutrient deficiencies. Makhoul and his colleagues reported that national policies and economic levels were important factors affecting anemia in pregnant women [18]. Since 2009, many countries have started to distribute folic acid to women of childbearing age for free [17, 19]. 83.3% of the patients in this study complained about folic acid supplementation before or in the first trimester. However, no pregnant women were found to be deficient in plasma folic acid or red blood cell folic acid during the study period, suggesting that this measure had achieved remarkable consequences in the study area and enhanced the period of childbearing age. Women's awareness of supplementing folic acid is of great significance to improve nutrition of pregnant women and prevent neural tube defects and anemia [20].

The results also demonstrated that the incidences of deficiency of vitamin A, borderline vitamin A, iron, and vitamin B12 in early pregnancy were 12.0%, 43.6%, 5.6%, and 0.4%, respectively. Further, the plasma vitamin A and iron concentrations between the anemia group and the non-anemia group showed statistically significant difference, suggesting that iron and vitamin A were still the main risk factors for anemia in the local population and should not be ignored. Our previous study found that plasma vitamin A had not only an important impact on immune and neurological functions but also a close correlation with anemia, which is consistent with the findings of this study. It is recommended that special attention should be paid to the monitoring of plasma vitamin A and iron concentrations for pregnancy anemia to ensure maternal and infant health [18, 19].

Conclusion

The preventive measures of "three early" play an important role in preventing and treating anemia during pregnancy. The incidence of anemia in pregnancy in the population of studied area is high, and the deficiency of nutrients related to anemia is mainly vitamin A and iron. Those factors need special consideration in nutrient supplementation plans by strengthening dietary nutrition guidance for pregnant women, improving nutritional status during pregnancy, and promoting maternal and infant health [20].

References

1. Zillmer K, Pokharel A, Spielman K, Kershaw M, Ayele K, Kidane Y, *et al*. 2017. Predictors of anemia in pregnant women residing in rural areas of the Oromiya region of Ethiopia. BMC Nutr. 3(1):1-8.
2. Zhang Q, Li Z, Ananth C. 2009. Prevalence and risk factors for anemia in pregnant women: a population-based prospective cohort study in China. Pediatr Perinat Epidemiol. 23(4):282-291.
3. Rashid S, Meier V, Patrick H. 2021. Review of Vitamin B12 deficiency in pregnancy: a diagnosis not to miss as veganism and vegetarianism become more prevalent. Eur J Haematol. 106:450-455.
4. Suchdev PS, Ruth LJ, Woodruff BA, Mbakaya C, Mandava U, Flores-Ayala R, *et al*. 2021. Selling sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: A cluster-randomized controlled trial. Am J Clin Nutr. 95(5):1223-1230.
5. Fang H, Guo Q, Ju L, Li S, Xu X, Piao W, *et al*. 2021. Weight status and self-perception of weight among women of childbearing age — China, 2015. China CDC Wkly. 3(9):185.
6. Patterson AJ, Brown WJ, Roberts DCK. 2001. Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. J Am Coll Nutr. 20(4):337-342.
7. Almawlah YH. 2017. Antibacterial activity of Punica granatum, Allium sativum and Piper nigrum against methicillin resistant *Staphylococcus aureus* (MRSA) isolated from wound infections in Al-Hilla , Iraq. Int J Chemtech Res. 10(2):188-190.
8. Mawlah Y, Naji M, Imari M, Abdulabbas H. 2022. Micro-RNA evaluation, specification, and stabilization study in mixed/non-mixed body fluids as a specific molecular marker. J Adv Biotechnol Exp Ther. 5(2):347.
9. Knoppert DC, Page A, Warren J, Seabrook JA, Carr M, Angelini M, *et al*. 2013. The effect of two different domperidone doses on maternal milk production. J Hum Lact. 29(1):38-44.
10. Dreyfuss ML, Stoltzfus RJ, Shrestha JB, Pradhan EK, LeClerq SC, Khatry SK, *et al*. 2000. Hookworms, malaria and vitamin a deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr Biochem. 130(10):2527-2536.
11. Ahmed F, Al-Sumaie MA. 2011. Risk factors associated with anemia and iron deficiency among Kuwaiti pregnant women. Int J Food Sci Nutr. 62(6):585-592.
12. Miner JC, Bisse E, Aebischer CP, Bell A, Wieland H, Lutschg J. 2000. Assessment of vitamin B-12, folate, and vitamin B-6 status and relation to sulfur amino acid metabolism in neonates. Am J Clin Nutr. 72(3):751-757.
13. Hao L, Ma J, Stampfer MJ, Ren A, Tian Y, Tang Y, *et al*. 2003. Geographical, seasonal and gender differences in folate status among Chinese adults. J Nutr. 133(11):3630-3635.
14. Hashim A, Harbi S, Burhan M, MAWLAH Y, Hadi A. 2023. Histological and physiological determinants of hypothyroidism in patients and its relationship with lipid profile. J Adv Biotechnol Exp Ther. 6(1):9-16.
15. Al-Mawlah YH, Alasadi YF, Al-Darraji MN. 2021. Association between genetic polymorphisms of (Cu/ZnSOD and CAT C262T) and the risk of breast cancer. Gene Rep. 25:101401.
16. Morchiladze N, Tkeshelashvili B, Gagua T, Gagua D. 2017. Prognostic risk of obstetric and perinatal complications in pregnant women with thyroid dysfunction. Georgian Med News. 264:5-21.
17. Makhoul Z, Taren D, Duncan B, Pandey P, Thomson C, Winzerling J, *et al*. 2012. Risk factors associated with anemia, iron deficiency and iron deficiency anemia in rural Nepali pregnant women. Southeast Asian J Trop Med Public Health. 43(3):735.
18. Abbasi A, Arooj S, Hussain W, Mughal AI, Habib N, Aziz W, *et al*. 2013. Causes of anemia in pregnant women of the state of azad kashmir: A cross-sectional survey. Health. 05(1):22-31.
19. Al-Mawlah YH, Al-Darraji MN, Al-Imari MJ. 2022. Study of small non-coding RNA (miRNA) expression pattern of fertile/infertile male semen. Acta Inform Med. 30(3):205–212.
20. Furness D, Fenech M, Dekker G, Khong TY, Roberts C, Hague W. 2013. Folate, Vitamin B12, Vitamin B6 and homocysteine: Impact on pregnancy outcome. Matern Child Nutr. 9(2):155-166.