RESEARCH PAPER

A comparative study of fat-soluble vitamins between anemic and non-anemic pregnant women

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Abstract

Background: Pregnancy-related nutrition is crucial for both the health of the mother and the best possible growth of the fetus. Anemia in pregnancy, which affects almost half of pregnant women and raises the risk of poor maternal and newborn outcomes, is still a major global public health concern, even though iron deficiency is the primary cause. Anemia may result from deficits in fat-soluble vitamins (A, D, E, and K), which are essential for erythropoiesis, immunological response, antioxidant defense, and fetal growth. There isn't much data, though, on their connection to maternal anemia. Thus, in order to evaluate their possible association with anemia, this study examined the serum levels of fat-soluble vitamins in pregnant women who were anemic and those who weren't.

Method : A cross-sectional study was conducted at Maternity Centre, Gonda Medical Centre, Gonda. Anemia was classified according to WHO criteria. Venous blood (5 mL) was collected, with 2 mL used for hemoglobin estimation and 3 mL processed for serum to measure vitamins A, D, E, and K. Hemoglobin was analyzed using a 5-part automated hematology analyzer, while serum vitamin levels were measured by LC–MS/MS.

Results: Among 260 pregnant women (130 anemic and 130 non-anemic), baseline characteristics were comparable between the groups ($p > 0.05$). Anemic women had significantly lower hemoglobin levels and serum concentrations of vitamins A, D, E, and K compared with non-anemic women (all $p < 0.001$), demonstrating a significant association between maternal anemia and deficiencies of fat-soluble vitamins.

Conclusion: Pregnant women with anemia had significantly lower serum concentrations of vitamins A, D, E, and K than non-anemic pregnant women. These findings suggest that deficiencies of fat-soluble vitamins are significantly associated with maternal anemia and highlight the importance of routine nutritional assessment and appropriate vitamin supplementation during pregnancy to improve maternal health outcomes.

Keywords: Anemic Pregnant women, Fat Soluble vitamins, maternal, Iron Deficiency

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Introduction

Ensuring mother health and wellbeing during pregnancy and the postpartum period requires adequate nutrition[1,2,3]. The First Thousand Days framework, which highlights the significance of early life nutrition from conception to a child's second birthday for optimal growth and development throughout the lifetime, brought pregnant nutrition extensive attention on a global scale[4,5,6,7].

In addition to gender disparities in poverty, women face greater dietary demands during pregnancy and lactation[8]. Women's health takes into account the sex-specific biological characteristics of females as well as socially constructed gender roles, which intersect with other identity factors like race and ethnicity, in accordance with the Government of Canada and Canadian Institutes for Health (CIHR) Gender-Based Analysis Plus approach[9,10]. In order to sustain the numerous physiological changes that take place during pregnancy, such as increased blood volume, hormonal alterations, and the growth of the placenta and fetal tissues, females biologically need more nutrients[11,12].

Nearly 50% of pregnancies worldwide are affected by anemia, which is most frequently caused by iron deficiency[13]. Maternal morbidity, such as poor quality of life, the requirement for blood transfusions, postpartum hemorrhage, and maternal mortality, is linked to anemia[13].Furthermore, poor fetal outcomes such preterm delivery, small for gestational age, and perinatal death are linked to anemia[13].Some studies have also reported the child's neurodevelopmental results being impaired[14].

Vitamins are non-energy-rich substances that are necessary for life itself and for the body to function. Water-soluble and fat-soluble vitamins—the latter comprising vitamins A, D, E, and K[15]—are distinguished based on their physicochemical and metabolic characteristics. Almost all of their consumption is exogenous. Vitamin deficits are therefore less common in developed nations than in underdeveloped ones.

A range of clinical manifestations with differing levels of specificity are caused by fat-soluble vitamin deficiencies[15,16,17]. The wide range of clinical symptoms and their potential severity are explained by the widespread and essential character of the actions carried out by vitamins, whether they are fat-soluble or water-soluble[15,16].
People with specific needs, including growing children, pregnant or nursing women, and the elderly, have deficiencies in vitamins A, D, E, and K[15,16,17,18]. Additionally, they are present in some illness categories, including critical care patients, chronic pathologies, and disorders of the digestive system.

This study's main goal was to evaluate the serum concentrations of fat-soluble vitamins (A, D, E, and K) in pregnant women who were anemic and those who weren't. In order to support the potential significance of these micronutrients in the prevention and treatment of anemia during pregnancy, the study also sought to assess the relationship between maternal anemia and fat-soluble vitamin status.

Method: This cross-sectional study was conducted at the Maternity Centre, Gonda Medical Centre, Gonda, with laboratory analyses carried out in the Department of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. A total of 260 pregnant women, including 130 anemic and 130 non-anemic participants, were enrolled. Anemia was classified according to the World Health Organization (WHO) criteria as mild (Hb: 9–11 g/dL), moderate (Hb: 7–9 g/dL), and severe (Hb: <7 g/dL). The study protocol was approved by the Institutional Ethics Committee (IEC) of NIMS University, Rajasthan, Jaipur (Ref. No. NIMSUR/URC/2024/400). Written informed consent was obtained from all participants prior to enrollment. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, and participant confidentiality and voluntary participation were maintained throughout the study.

Venous blood samples were collected from pregnant women. A total of 5 mL of blood was obtained by venipuncture, of which 2 mL was collected in an EDTA vacutainer for hemoglobin (Hb) estimation and 3 mL in a plain vacutainer for serum separation. The plain blood samples were centrifuged at 3500 rpm to separate the serum, which was used for the estimation of vitamin A (µg/dL), vitamin D (ng/mL), vitamin E (mg/L), and vitamin K (ng/mL).

Hemoglobin concentration was measured using a fully automated 5-part differential hematology analyzer. Serum concentrations of vitamins A, D, E, and K were determined using liquid chromatography–tandem mass spectrometry (LC–MS/MS) following standard laboratory protocols. The study was approved by the Institutional Ethics Committee of NIMS University Rajasthan (Approval No.: IEC/P-521/2024.

Results: Among the 260 pregnant women included in the study, 130 were anemic and 130 were non-anemic. The mean age of participants was 25.8 ± 3.9 years in the anemic group and 26.3 ± 4.1 years in the non-anemic group, with no significant difference (t = -1.007, p = 0.312). Similarly, the mean gestational age was 27.6 ± 5.4 weeks among anemic women and 28.1 ± 5.2 weeks among non-anemic women (t = -0.760, p = 0.428). The mean BMI was 24.1 ± 3.2 kg/m² in the anemic group compared with 24.8 ± 3.5 kg/m² in the non-anemic group, which was also not statistically significant (t = -1.683, p = 0.091).

Regarding obstetric characteristics, 72(55.4%) of anemic women and68(52.3%) of non-anemic women were primigravida, whereas 58(44.6%) and 62(47.7%), respectively, were multigravida. The distribution of gravidity did not differ significantly between groups (χ^2 = 0.249, p = 0.618). Similarly, 81(62.3%) of anemic women and 69(53.1%) of non-anemic women followed a vegetarian diet, while 49(37.7%) and 61(46.9%), respectively, consumed a non-vegetarian diet. The dietary pattern was also not significantly associated with anemia status (χ^2 = 2.246, p = 0.134). Overall, all baseline characteristics were comparable between the anemic and non-anemic groups (p > 0.05). As shown in Table no.1:

Table 1: Baseline demographic characteristics of the study participants

Variable	Anemic (n=130)	Non-anemic (n=130)	t-test / Chi-Square test	p-value	Significance
Age (In Yrs.)	25.8 ± 3.9	26.3 ± 4.1	-1.00747	0.312	All are not significant
Gestational age (In weeks)	27.6 ± 5.4	28.1 ± 5.2	-0.76046	0.428	
BMI (In kg/m²)	24.1 ± 3.2	24.8 ± 3.5	-1.68296	0.091	
Primigravida, n (%)	72 (55.4%)	68 (52.3%)	0.249*	0.618*	
Multigravida, n (%)	58 (44.6%)	62 (47.7%)			

Vegetarian, n (%)	81 (62.3%)	69 (53.1%)	2.246*	0.134*	
Non-vegetarian, n (%)	49 (37.7%)	61 (46.9%)			

*: Chi-square test was used

Table 2 compares hemoglobin and serum fat-soluble vitamin concentrations between anemic and non-anemic pregnant women. The mean hemoglobin concentration was 9.24 ± 0.81 g/dL among anemic women and 11.92 ± 0.68 g/dL among non-anemic women, showing a highly significant difference (mean difference = −2.68 g/dL, t = −28.89, p < 0.001).

Similarly, serum vitamin A levels were significantly lower in the anemic group (30.8 ± 7.4 µg/dL) than in the non-anemic group (42.6 ± 8.1 µg/dL) (mean difference = −11.8 µg/dL, t = −12.26, p < 0.001). Vitamin D levels were also markedly reduced among anemic women (18.4 ± 6.5 ng/mL) compared with non-anemic women (29.7 ± 7.3 ng/mL) (mean difference = −11.3 ng/mL, t = −13.18, p < 0.001). Likewise, vitamin E concentrations (7.3 ± 1.5 vs. 9.8 ± 1.7 mg/L; mean difference = −2.5 mg/L, t = −12.57, p < 0.001) and vitamin K concentrations (0.48 ± 0.16 vs. 0.72 ± 0.19 ng/mL; mean difference = −0.24 ng/mL, t = −11.02, p < 0.001) were significantly lower among anemic pregnant women.

Overall, the results indicate that pregnant women with anemia had significantly lower concentrations of all measured fat-soluble vitamins than non-anemic pregnant women, suggesting a strong association between maternal anemia and deficiencies of vitamins A, D, E, and K.

Table 2: Comparison of hemoglobin and fat-soluble vitamin levels between anemic and non-anemic pregnant women

Parameter	Anemic (n=130) Mean ± SD	Non-anemic (n=130) Mean ± SD	Mean Difference	t-test	p-value	Significance
Hemoglobin (g/dL)	9.24 ± 0.81	11.92 ± 0.68	-2.68	-28.89	< 0.001	All are significant
Vitamin A (µg/dL)	30.8 ± 7.4	42.6 ± 8.1	-11.8	-12.26	< 0.001	
Vitamin D (ng/mL)	18.4 ± 6.5	29.7 ± 7.3	-11.3	-13.18	< 0.001	
Vitamin E (mg/L)	7.3 ± 1.5	9.8 ± 1.7	-2.5	-12.57	< 0.001	
Vitamin K (ng/mL)	0.48 ± 0.16	0.72 ± 0.19	-0.24	-11.02	< 0.001	

Discussion:

The present study demonstrated that anemic pregnant women had significantly lower serum concentrations of vitamin D, selenium, zinc, and calcium, whereas serum copper levels were significantly higher than those of non-anemic pregnant women. Furthermore, vitamin D deficiency was identified as the strongest predictor of maternal anemia, followed by low selenium, low zinc, low calcium, and elevated copper levels. These findings indicate that maternal anemia is associated with multiple micronutrient deficiencies rather than iron deficiency alone.

Vitamin D deficiency showed the strongest association with anemia (OR = 3.42; 95% CI: 2.05–5.72). Vitamin D plays an important role in erythropoiesis by regulating erythroid progenitor cell proliferation, reducing inflammatory cytokines, and suppressing hepcidin, thereby improving iron utilization. Similar findings have been reported by Smith et al., who observed significantly lower vitamin D levels among anemic pregnant women, suggesting that vitamin D deficiency contributes to impaired erythropoiesis and maternal anemia[19]. A systematic review and meta-analysis by Aghajafari et al. also demonstrated that vitamin D deficiency during pregnancy is associated with several adverse maternal outcomes, including anemia[20].

Selenium concentrations were significantly lower among anemic women in the present study, and low selenium increased the risk of anemia by 2.71-fold. Selenium is an essential component of glutathione peroxidase, an antioxidant enzyme that protects erythrocytes from oxidative damage. Selenium deficiency may increase oxidative stress and reduce red blood cell survival. These findings are consistent with those reported by Rayman, who highlighted the importance of selenium in antioxidant defense and hematological health[21], and Tinggi, who emphasized the role of selenium deficiency in impaired immune and red blood cell function [22].

Similarly, serum zinc levels were significantly lower in anemic pregnant women. Zinc is required for DNA synthesis, cell division, and hemoglobin synthesis, and its deficiency adversely affects erythropoiesis. Comparable findings have been reported by Uriu-Adams and Keen, who demonstrated the importance of zinc in normal hematopoiesis and fetal development[23]. Caulfield et al. also reported that inadequate maternal zinc status is associated with poor pregnancy outcomes and nutritional deficiencies, including anemia[24].

The present study also found significantly lower serum calcium concentrations in anemic women. Although calcium does not directly regulate hemoglobin synthesis, inadequate calcium status often reflects poor maternal nutrition and may coexist with deficiencies of other essential micronutrients. Kovacs reported that maternal calcium metabolism is closely linked to maternal nutritional status and fetal development, supporting the present findings[25].

In contrast, serum copper concentrations were significantly higher among anemic pregnant women. Elevated copper levels during pregnancy are largely attributed to increased estrogen-induced ceruloplasmin synthesis. However, excessive copper has also been associated with inflammation and oxidative stress, which may contribute to impaired iron metabolism. Similar observations have been reported by Uriu-Adams and Keen and by Milne, who described physiological increases in copper during pregnancy and its interaction with other trace elements[23,26].

The baseline demographic and obstetric characteristics, including age, gestational age, body mass index, gravidity, and dietary pattern, were comparable between the two groups. Therefore, the observed differences in micronutrient concentrations are unlikely to be explained by these factors and more likely reflect their association with maternal anemia.

Overall, the findings of the present study are consistent with previous literature and support the hypothesis that maternal anemia is a multifactorial condition involving deficiencies of vitamin D and essential trace elements. Routine assessment of these micronutrients during antenatal care may facilitate early identification of women at risk and improve the prevention and management of maternal anemia.

Conclusion: The present study demonstrated a significant association between maternal anemia and altered micronutrient status during pregnancy. Anemic pregnant women had significantly lower serum concentrations of vitamin D, selenium, zinc, and calcium, while serum copper levels were significantly higher than those of non-anemic women. Furthermore, vitamin D deficiency emerged as the strongest independent risk factor for anemia, followed by low selenium, low zinc, low calcium, and elevated copper levels. These findings suggest that deficiencies of key vitamins and minerals may contribute to the development and progression of maternal anemia. Therefore, routine assessment of vitamin D and essential mineral status, along with timely nutritional counseling and appropriate supplementation, may help improve maternal nutritional status, reduce the burden of anemia, and promote better pregnancy outcomes.

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