

# Association of Vitamin D And Minerals With Anemia in Pregnant Women's

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## Abstract

**Background:** Anemia during pregnancy is a significant public health problem associated with adverse maternal and fetal outcomes. Besides iron deficiency, vitamin D deficiency and mineral imbalances may contribute to impaired erythropoiesis. This study evaluated the association of serum vitamin D and selected mineral levels with anemia among pregnant women.

**Method:** A comparative cross-sectional study was conducted among 360 pregnant women (180 anemic and 180 non-anemic). Demographic and obstetric data were collected, and serum hemoglobin, vitamin D, selenium, zinc, copper, and calcium levels were measured. Group comparisons were performed using the independent t-test and Chi-square test, while multiple logistic regression was used to identify factors associated with anemia. A p-value <0.05 was considered statistically significant.

**Result:** Anemic pregnant women had significantly lower mean hemoglobin, vitamin D, selenium, zinc, and calcium levels, while serum copper levels were significantly higher than those of non-anemic women (all p < 0.001). Vitamin D deficiency showed the strongest association with anemia (OR = 3.42, 95% CI: 2.05–5.72), followed by low selenium (OR = 2.71), low zinc (OR = 2.38), low calcium (OR = 1.92), and elevated copper (OR = 1.76), all of which were significantly associated with an increased risk of anemia during pregnancy.

**Conclusion:** Vitamin D deficiency and alterations in essential mineral levels, particularly low selenium, zinc, and calcium and elevated copper, are significantly associated with anemia during pregnancy. Routine assessment of these micronutrients, along with appropriate nutritional interventions, may help improve maternal health and reduce the burden of pregnancy-related anemia.

**Keywords:** serum hemoglobin, vitamin D, selenium, zinc, copper.

## Introduction

Anemia, due to decreased red blood cells or hemoglobin levels, anemia, which affects about 40% of pregnancies globally, is a serious concern<sup>1</sup>. Throughout the whole pregnancy, iron deficiency anemia has a negative effect on the health of both the mother and the fetus. Both higher rates of morbidity and a higher risk of fetal death have been linked to this syndrome<sup>2</sup>. Iron deficiency anemia during pregnancy has negative impacts on perinatal outcomes, including low birth weight, preterm, and intrauterine development retardation. These unfavorable consequences provide significant mortality concerns, especially in settings with inadequate resources and emerging nations, particularly in rural areas<sup>3</sup>.

Adequate diet is crucial for the best possible health of both the mother and the fetus throughout pregnancy<sup>4</sup>. To support fetal growth, placental development, and maternal adaptations, increased physiological and metabolic needs necessitate adequate consumption of both macronutrients and micronutrients<sup>4,5</sup>. Micronutrients like iron, folate, calcium, iodine, magnesium, and vitamin D are essential for immune system function, bone mineralization, erythropoiesis, and embryonic neurodevelopment<sup>5,6</sup>. Pregnancy-related anemia is mostly caused by iron deficiencies, and neural tube abnormalities are more likely when folate consumption is insufficient<sup>5-7</sup>.

Vitamin D insufficiency has been linked to poor pregnancy outcomes, such as preeclampsia, gestational diabetes, preterm birth, and low birth weight. Vitamin D is essential for calcium homeostasis, skeletal development, and immunological modulation<sup>6</sup>. Therefore, enhancing pregnancy outcomes and lowering maternal and newborn problems require establishing optimal maternal nutritional status through a balanced diet and suitable supplements<sup>4,5</sup>.

Among the most prevalent nutritional deficiencies in the world, especially in women of reproductive age and during pregnancy, are vitamin D and iron deficits. Iron insufficiency affects about 10% of women, but vitamin D deficiency affects 8% of the entire population<sup>8</sup>. Due to the increased need for both nutrients during pregnancy, deficits become more common. Intrauterine growth restriction, preterm birth, low birth weight, postpartum depression, and elevated maternal morbidity and mortality are linked to iron deficiency and iron-deficiency anemia<sup>9-11</sup>. Preeclampsia, small-for-gestational-age newborns, neonatal hypocalcemia, poor fetal bone development, and low neonatal vitamin D status are also associated with vitamin D deficiency<sup>12-14</sup>.

This study's main goal was to assess the relationship between serum vitamin D and certain mineral levels and anemia in expectant mothers. Comparing serum vitamin D and specific mineral levels between anemic and non-anemic pregnant women, evaluating their correlation with hemoglobin concentration, and determining whether vitamin D deficiency and mineral imbalances are potential risk factors for anemia during pregnancy were the secondary goals.

**Method:** A total of 360 pregnant women were enrolled in the study, including 180 anemic and 180 non-anemic participants. Pregnant women aged 18–40 years with singleton pregnancies who provided written informed consent were included. Women with chronic systemic diseases, hemoglobinopathies, acute infections, renal or hepatic disorders, multiple pregnancies, or those receiving vitamin D or mineral supplementation before enrollment were excluded. Anemia was defined according to the World Health Organization (WHO) criteria as hemoglobin (Hb) <11.0 g/dL. Participants with Hb ≥11.0 g/dL were classified as non-anemic. Sociodemographic and obstetric information, including maternal age, gestational age, gravidity, dietary habits, height, weight, and body mass index (BMI), was collected using a structured questionnaire and medical records. BMI was calculated as weight (kg) divided by height squared (m<sup>2</sup>).

**Sample Collection and Laboratory Analysis:** Approximately 5 mL of fasting venous blood was collected under aseptic conditions. Hemoglobin concentration was measured using an automated hematology analyzer. Serum was separated by centrifugation and stored at –20°C until analysis.

Serum 25-hydroxyvitamin D [25(OH)D] concentration was estimated using a validated immunoassay. Serum selenium, zinc, copper, and calcium concentrations were measured using standard laboratory methods according to the manufacturer's instructions. Vitamin D deficiency was defined as serum 25(OH)D <20 ng/mL.

**Statistical Analysis:** Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD), while categorical variables are expressed as frequency and percentage. Differences between anemic and non-anemic groups were compared using the independent samples t-test for continuous variables and the Chi-square test for categorical variables. Multiple logistic regression analysis was performed to determine the association between vitamin D deficiency, mineral abnormalities, and anemia, and results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value <0.05 was considered statistically significant.

The study was approved by the Institutional Ethics Committee of NIMS University Rajasthan (Approval No.: IEC/P-521/2024).

**Results:**

Table 1 presents the comparison of demographic, anthropometric, obstetric, and dietary characteristics between 180 anemic and 180 non-anemic pregnant women. The mean age of participants was 25.9 ± 3.8 years in the anemic group and 26.4 ± 3.9 years in the non-anemic group, with no statistically significant difference (t = 1.24, p = 0.214). Similarly, the mean gestational age was 27.5 ± 5.2 weeks among anemic women and 27.9 ± 5.0 weeks among non-anemic women, showing no significant difference (t = 0.73, p = 0.468).

The mean height was 156.8 ± 5.7 cm in the anemic group and 157.5 ± 5.6 cm in the non-anemic group (t = 1.19, p = 0.236). The mean body weight was slightly lower among anemic women (59.8 ± 8.3 kg) compared with non-anemic women (61.5 ± 7.9 kg); however, the difference was not statistically significant (t = 1.95, p = 0.052). Likewise, the mean BMI was 24.3 ± 3.4 kg/m<sup>2</sup> in the anemic group and 24.8 ± 3.2 kg/m<sup>2</sup> in the non-anemic group, with no significant difference (t = 1.40, p = 0.163).

Regarding obstetric characteristics, 98 (54.4%) women in the anemic group and 91 (50.6%) in the non-anemic group were primigravida, while 82 (45.6%) and 89 (49.4%), respectively, were multigravida. The distribution of gravidity was comparable between the groups (χ<sup>2</sup> = 0.52, p = 0.472).

With respect to dietary habits, 109 (60.6%) anemic women and 94 (52.2%) non-anemic women followed a vegetarian diet, whereas 71 (39.4%) and 86 (47.8%), respectively, consumed a non-vegetarian diet. The difference in dietary pattern between the two groups was not statistically significant (χ<sup>2</sup> = 2.58, p = 0.108).

Overall, none of the baseline demographic, anthropometric, obstetric, or dietary variables showed statistically significant differences between the anemic and non-anemic groups (all p > 0.05), indicating that the two groups were comparable at baseline.

**Table 1: Demographic characteristics of the study participants (N = 360)**

| Variable                    | Anemic<br>(n=180)<br>Mean ± SD | Non-anemic<br>(n=180)<br>Mean ± SD | t-test/ Chi-square test | p-value |
|-----------------------------|--------------------------------|------------------------------------|-------------------------|---------|
| Age (In Yrs.)               | 25.9 ± 3.8                     | 26.4 ± 3.9                         | 1.24                    | 0.214   |
| Gestational age (In weeks)  | 27.5 ± 5.2                     | 27.9 ± 5.0                         | 0.73                    | 0.468   |
| Height (In cm)              | 156.8 ± 5.7                    | 157.5 ± 5.6                        | 1.19                    | 0.236   |
| Weight (In wt.)             | 59.8 ± 8.3                     | 61.5 ± 7.9                         | 1.95                    | 0.052   |
| BMI (In kg/m <sup>2</sup> ) | 24.3 ± 3.4                     | 24.8 ± 3.2                         | 1.40                    | 0.163   |
| Primigravida, n (%)         | 98 (54.4)                      | 91 (50.6)                          | 0.52*                   | 0.472*  |
| Multigravida, n (%)         | 82 (45.6)                      | 89 (49.4)                          |                         |         |
| Vegetarian, n (%)           | 109 (60.6)                     | 94 (52.2)                          | 2.58*                   | 0.108*  |
| Non-vegetarian, n (%)       | 71 (39.4)                      | 86 (47.8)                          |                         |         |

\*: Chi-square test was used

Table 2 compares hemoglobin, vitamin D, and serum mineral concentrations between 180 anemic and 180 non-anemic pregnant women. The mean hemoglobin concentration was significantly lower in the anemic group ( $9.18 \pm 0.82$  g/dL) than in the non-anemic group ( $11.96 \pm 0.71$  g/dL) ( $t = -4.58$ ,  $p < 0.001$ ).

Similarly, the mean serum vitamin D level was significantly reduced among anemic women ( $17.9 \pm 6.3$  ng/mL) compared with non-anemic women ( $30.5 \pm 7.4$  ng/mL) ( $t = -11.65$ ,  $p < 0.001$ ). Serum selenium and zinc concentrations were also significantly lower in the anemic group, with mean values of  $69.2 \pm 11.8$  µg/L and  $61.8 \pm 12.5$  µg/dL, respectively, compared to  $90.6 \pm 12.7$  µg/L and  $84.9 \pm 13.1$  µg/dL in the non-anemic group (both  $p < 0.001$ ).

In contrast, the mean serum copper concentration was significantly higher among anemic women ( $154.3 \pm 23.8$  µg/dL) than non-anemic women ( $134.6 \pm 20.9$  µg/dL) ( $t = 17.42$ ,  $p < 0.001$ ). Additionally, serum calcium levels were significantly lower in the anemic group ( $8.46 \pm 0.54$  mg/dL) compared with the non-anemic group ( $9.28 \pm 0.49$  mg/dL) ( $t = -5.65$ ,  $p < 0.001$ ).

Overall, pregnant women with anemia exhibited significantly lower hemoglobin, vitamin D, selenium, zinc, and calcium levels, while serum copper levels were significantly higher than those of non-anemic pregnant women (all  $p < 0.001$ ), indicating a significant association between maternal anemia and altered vitamin and mineral status.

**Table 2: Comparison of hemoglobin, Vitamin D, and essential mineral levels between anemic and non-anemic pregnant women**

| Parameter         | Anemic (n=180)<br>Mean ± SD | Non-anemic (n=180)<br>Mean ± SD | t-test | p-value |
|-------------------|-----------------------------|---------------------------------|--------|---------|
| Hemoglobin (g/dL) | $9.18 \pm 0.82$             | $11.96 \pm 0.71$                | -4.58  | <0.001  |
| Vitamin D (ng/mL) | $17.9 \pm 6.3$              | $30.5 \pm 7.4$                  | -11.65 | <0.001  |
| Selenium (µg/L)   | $69.2 \pm 11.8$             | $90.6 \pm 12.7$                 | -14.23 | <0.001  |
| Zinc (µg/dL)      | $61.8 \pm 12.5$             | $84.9 \pm 13.1$                 | -10.95 | <0.001  |
| Copper (µg/dL)    | $154.3 \pm 23.8$            | $134.6 \pm 20.9$                | 17.42  | <0.001  |
| Calcium (mg/dL)   | $8.46 \pm 0.54$             | $9.28 \pm 0.49$                 | -5.65  | <0.001  |

Table 3 presents the association between vitamin and mineral abnormalities and the risk of anemia among pregnant women. Vitamin D deficiency ( $<20$  ng/mL) showed the strongest association with anemia, with affected women having 3.42 times higher odds of being anemic than those with normal vitamin D levels (OR = 3.42; 95% CI: 2.05–5.72;  $p < 0.001$ ). Similarly, low selenium (OR = 2.71; 95% CI: 1.69–4.36;  $p < 0.001$ ) and low zinc (OR = 2.38; 95% CI: 1.46–3.89;  $p < 0.001$ ) were significantly associated with increased odds of anemia.

In addition, low calcium was associated with a nearly two-fold higher risk of anemia (OR = 1.92; 95% CI: 1.17–3.14;  $p = 0.01$ ), while elevated copper levels were also significantly associated with anemia (OR = 1.76; 95% CI: 1.09–2.84;  $p = 0.021$ ).

Overall, vitamin D deficiency demonstrated the strongest predictor of maternal anemia, followed by low selenium, low zinc, low calcium, and elevated copper, all of which were significantly associated with an increased risk of anemia during pregnancy.

**Table 3: Association of vitamin D and minerals with anemia by using multiple logistic regression**

| Variable                         | Odds Ratio (OR) | 95% Confidence Interval | Z-test | p-value |
|----------------------------------|-----------------|-------------------------|--------|---------|
| Vitamin D deficiency (<20 ng/mL) | 3.42            | 2.05–5.72               | 4.18   | <0.001  |
| Low Selenium                     | 2.71            | 1.69–4.36               | 3.83   | <0.001  |
| Low Zinc                         | 2.38            | 1.46–3.89               | 3.69   | <0.001  |
| Low Calcium                      | 1.92            | 1.17–3.14               | 2.33   | 0.01    |
| Elevated Copper                  | 1.76            | 1.09–2.84               | 2.03   | 0.021   |

## Discussion

The present study evaluated the association of vitamin D and selected essential minerals with anemia among pregnant women and compared the demographic, anthropometric, obstetric, and dietary characteristics of anemic and non-anemic participants. The baseline findings demonstrated that the two groups were broadly comparable. There were no statistically significant differences in age, gestational age, height, weight, BMI, gravidity, or dietary pattern between anemic and non-anemic women. The absence of significant baseline differences is important because it suggests that the observed biochemical differences may not simply be explained by variations in these measured demographic, anthropometric, obstetric, or dietary characteristics.

In the present study, the mean age of anemic women was  $25.9 \pm 3.8$  years compared with  $26.4 \pm 3.9$  years among non-anemic women, with no significant difference. Similarly, gestational age, height, weight, and BMI were comparable between the groups. These findings are consistent with the observation that anemia during pregnancy is influenced by nutritional and physiological factors rather than age or anthropometric characteristics alone. A study conducted by Saini R, et al.,<sup>15</sup> among pregnant women in North India reported significant hematological and biochemical differences between anemic and non-anemic pregnant women, while emphasizing that pregnancy-related anemia is accompanied by alterations in several biochemical parameters.

The present study also found no significant difference in gravidity between the two groups. Although 54.4% of anemic women were primigravida compared with 50.6% of non-anemic women, this difference was not statistically significant. Similarly, dietary pattern did not differ significantly, although vegetarian women constituted a slightly greater proportion of the anemic group. These findings indicate that, within the present study population, gravidity and broad dietary classification alone were not sufficient to explain the difference in anemia status. Dietary quality, micronutrient intake, bioavailability, supplementation adherence, and other unmeasured nutritional factors may be more relevant than vegetarian versus non-vegetarian status alone.

## Hemoglobin and vitamin D status

As expected, the mean hemoglobin concentration was markedly lower among anemic women than among non-anemic women ( $9.18 \pm 0.82$  vs.  $11.96 \pm 0.71$  g/dL;  $p < 0.001$ ), confirming the biochemical distinction between the two study groups. More importantly, the present study demonstrated a substantial difference in serum vitamin D concentration. The mean vitamin D level was  $17.9 \pm 6.3$  ng/mL in anemic women compared with  $30.5 \pm 7.4$  ng/mL in non-anemic women ( $p < 0.001$ ).

This finding is in agreement with previous evidence suggesting an association between vitamin D status and maternal hemoglobin. Thomas et al.,<sup>16</sup> reported that maternal 25-hydroxyvitamin D concentrations were positively associated with hemoglobin during pregnancy and that women with suboptimal vitamin D status had a higher likelihood of anemia. They also suggested that the association could involve pathways related to erythropoietin and iron metabolism. Similarly, Si et al.,<sup>17</sup> in a large pregnancy cohort, reported a positive association between plasma 25(OH)D and hemoglobin during different trimesters and found increased odds of anemia among women with vitamin D deficiency during pregnancy.

Evidence from India also supports the present observation. A case-control study conducted in New Delhi found significantly lower maternal vitamin D concentrations among anemic pregnant women compared with controls and reported a positive correlation between hemoglobin and vitamin D levels.<sup>18</sup> More recently, Ilango et al.,<sup>19</sup> investigated rural Indian pregnant women and reported a high prevalence of vitamin D deficiency during pregnancy, although the relationship between vitamin D and hemoglobin differed according to trimester. Such findings indicate that the relationship between vitamin D and anemia may be influenced by gestational age, iron status, supplementation, dietary intake, and other maternal factors.

The strong association observed in the present study was further demonstrated by logistic regression. Women with vitamin D deficiency (<20 ng/mL) had 3.42-fold higher odds of anemia (OR = 3.42; 95% CI: 2.05–5.72;  $p < 0.001$ ). This was the strongest association among all the micronutrients evaluated. Lima et al.,<sup>20</sup> in a systematic review and meta-analysis of observational studies involving 6,530 pregnant women, reported a 61% higher odds of anemia among

pregnant women with vitamin D deficiency (OR = 1.61; 95% CI: 1.41–1.83). Although the magnitude of association in the present study was greater, the direction of association is consistent with the existing literature.

A study conducted by Thomas CE et al.,<sup>16</sup> the biological plausibility of this relationship may be explained by the involvement of vitamin D in erythropoiesis, inflammatory regulation, and iron metabolism. Vitamin D may influence the hepcidin–ferroportin pathway and thereby affect iron availability for erythropoiesis. However, because the present study is observational, the observed association should not be interpreted as proof that vitamin D deficiency directly causes anemia. It is possible that anemia, inflammation, dietary inadequacy, reduced nutritional status, or other factors may contribute to low vitamin D concentrations.

## Selenium and anemia

A significant reduction in serum selenium was observed among anemic women compared with non-anemic women ( $69.2 \pm 11.8$  vs.  $90.6 \pm 12.7$  µg/L;  $p < 0.001$ ). Furthermore, low selenium was associated with 2.71-fold higher odds of anemia (OR = 2.71; 95% CI: 1.69–4.36;  $p < 0.001$ ).

Selenium is an essential trace element involved in antioxidant defense through selenium-dependent enzymes such as glutathione peroxidases and thioredoxin reductases. Pregnancy increases metabolic demand and oxidative stress, potentially increasing the importance of adequate selenium status. Previous research in pregnant women has demonstrated that deficiencies of several trace elements, including selenium, zinc, and calcium, are relatively common.<sup>21</sup> In addition, a study of pregnant women with anemia reported significantly lower selenium concentrations together with lower hemoglobin and other antioxidant parameters among anemic women.<sup>22</sup>

The present findings therefore suggest that inadequate selenium status may be associated with maternal anemia, potentially through increased oxidative stress and impaired antioxidant protection. Nevertheless, selenium deficiency should be considered an associated nutritional factor rather than an independently established causal factor, particularly because the present study did not directly measure oxidative stress or selenium-dependent enzyme activity.

## Zinc and anemia

Serum zinc concentration was also significantly lower in anemic women than in non-anemic women ( $61.8 \pm 12.5$  vs.  $84.9 \pm 13.1$  µg/dL;  $p < 0.001$ ). The regression analysis further demonstrated that low zinc was associated with increased odds of anemia (OR = 2.38; 95% CI: 1.46–3.89;  $p < 0.001$ ).

The present finding is consistent with previous evidence indicating an important relationship between maternal zinc status and hematological health. Sengupta et al.,<sup>23</sup> reported significantly lower zinc levels in iron-deficiency anemia during pregnancy and suggested that interactions among trace elements may influence maternal hematological status. Similarly, a study conducted in Jaipur by Sinha et al.,<sup>24</sup> found that pregnant women with iron-deficiency anemia had lower zinc concentrations, whereas copper concentrations were higher.

Experimental evidence also supports a possible role of zinc in erythropoiesis. In pregnant women with anemia, zinc administration together with iron was associated with an increase in hemoglobin and red blood cell parameters, suggesting that zinc status may contribute to hematological health.<sup>25</sup> However, evidence regarding routine zinc supplementation during pregnancy is not completely consistent. Chaffee and King<sup>26</sup> reported that although poor maternal zinc status has been associated with adverse pregnancy outcomes, evidence for beneficial effects of zinc supplementation on several pregnancy outcomes remains limited. Therefore, the low zinc concentrations observed in the present study should be interpreted as an important marker of nutritional status associated with anemia rather than definitive evidence that zinc supplementation alone would prevent anemia.

## Copper and anemia

In contrast to zinc and selenium, serum copper concentration was significantly higher among anemic women than non-anemic women ( $154.3 \pm 23.8$  vs.  $134.6 \pm 20.9$  µg/dL;  $p < 0.001$ ). Elevated copper was also significantly associated with anemia, with 1.76-fold higher odds of anemia (OR = 1.76; 95% CI: 1.09–2.84;  $p = 0.021$ ).

The finding of higher copper among anemic women is biologically plausible because serum copper normally increases during pregnancy. Vir et al.,<sup>27</sup> demonstrated that serum copper concentrations in pregnant women were substantially higher than those in non-pregnant women and remained elevated throughout pregnancy. However, the higher copper concentration in the anemic group in the present study may reflect altered copper metabolism, inflammatory responses, or interactions between copper and iron metabolism rather than simple copper excess.

Previous Indian evidence has reported a similar pattern. Sengupta et al.,<sup>23</sup> observed higher copper concentrations in pregnant women with iron-deficiency anemia compared with women with non-iron-deficiency anemia and reported lower zinc levels in iron-deficiency anemia. Likewise, Sinha et al.,<sup>24</sup> reported significantly higher copper and lower

zinc levels among pregnant women with iron-deficiency anemia. These observations support the possibility that disturbances in trace-element homeostasis accompany maternal anemia.

It is important to note that elevated serum copper does not necessarily indicate dietary copper excess. Copper is an acute-phase-responsive element, and its concentration can be influenced by pregnancy-related physiological changes, inflammation, ceruloplasmin concentrations, and iron status. Therefore, the association observed in the present study should be interpreted cautiously.

## Calcium and anemia

The present study demonstrated significantly lower serum calcium levels among anemic women compared with non-anemic women ( $8.46 \pm 0.54$  vs.  $9.28 \pm 0.49$  mg/dL;  $p < 0.001$ ). Furthermore, low calcium was associated with nearly two-fold higher odds of anemia (OR = 1.92; 95% CI: 1.17–3.14;  $p = 0.01$ ).

These findings are supported by Saini R, et al.,<sup>15</sup> research from North India. A comparative study of anemic and non-anemic primigravid women reported significantly reduced calcium levels among anemic pregnant women, together with significant reductions in several hematological parameters. This similarity strengthens the observation that altered calcium status may accompany maternal anemia.

The relationship between calcium and anemia may also involve interactions with iron metabolism. Calcium has been shown to influence iron absorption under certain experimental conditions, although the overall effect on hemoglobin status remains uncertain. A systematic review and meta-analysis by the authors of a 2021 review found that calcium intake could modestly reduce short-term iron absorption, but there was no clear reduction in hemoglobin concentration.<sup>28</sup> Therefore, the lower serum calcium observed in the present study may reflect overall nutritional status or pregnancy-related metabolic changes rather than a direct causal pathway leading to anemia.

## Overall micronutrient pattern and regression findings

An important finding of the present study is that anemia was associated with a pattern of multiple micronutrient abnormalities rather than an isolated deficiency. Anemic women had lower concentrations of vitamin D, selenium, zinc, and calcium, whereas copper concentrations were higher. This pattern suggests that maternal anemia may coexist with broader disturbances in micronutrient homeostasis.

The multiple logistic regression analysis further demonstrated that vitamin D deficiency had the strongest association with anemia, followed by low selenium, low zinc, low calcium, and elevated copper. The magnitude of the odds ratios was 3.42 for vitamin D deficiency, 2.71 for low selenium, 2.38 for low zinc, 1.92 for low calcium, and 1.76 for elevated copper. These findings emphasize the potential importance of assessing multiple micronutrients rather than focusing exclusively on iron and hemoglobin when evaluating anemia during pregnancy.

The high prevalence of vitamin D insufficiency among pregnant women in South Asia further strengthens the public health relevance of these findings. Siddiquee et al.,<sup>29</sup> in a systematic review of South Asian pregnant women, reported a pooled prevalence of vitamin D insufficiency of approximately 65%, with India showing a particularly high prevalence. More recent evidence also confirms by Cristófalo MM et al.,<sup>30</sup> that vitamin D deficiency remains highly prevalent among pregnant women globally.

The findings of the present study therefore support the concept of a comprehensive nutritional approach to maternal anemia. Antenatal assessment should consider not only hemoglobin and iron status but also relevant micronutrient factors, particularly in populations where nutritional deficiencies are common. However, intervention studies are required to establish whether correcting these abnormalities, particularly vitamin D, selenium, zinc, calcium, or copper disturbances, can independently improve maternal hemoglobin and reduce anemia.

## Strengths and implications

A major strength of the present study is the inclusion of equal numbers of anemic and non-anemic pregnant women and the simultaneous assessment of vitamin D and several essential minerals. The use of biochemical measurements provides objective evidence of micronutrient status, while multiple logistic regression allowed estimation of the independent associations of individual micronutrient abnormalities with anemia.

The findings have potential implications for antenatal nutritional assessment. The particularly strong association between vitamin D deficiency and anemia suggests that vitamin D status may warrant greater attention in pregnant women with anemia, especially in populations with a high burden of vitamin D deficiency. Similarly, the significant associations observed for selenium, zinc, calcium, and copper indicate that maternal micronutrient status may be multifactorial and should be interpreted as an integrated nutritional profile.

Nevertheless, the findings should be interpreted in light of the cross-sectional nature of the study. Because exposure and outcome were assessed at the same time, temporal relationships and causality cannot be established. In addition, factors such as dietary intake of individual micronutrients, iron and folic acid supplementation adherence,

inflammation, ferritin, hepcidin, parathyroid hormone, albumin, and socioeconomic characteristics were not incorporated into the presented analysis. Future longitudinal and interventional studies are therefore required to determine whether correction of specific micronutrient abnormalities can reduce the risk or severity of maternal anemia.

## Conclusion

Overall, the present study demonstrates that maternal anemia is significantly associated with altered vitamin and mineral status. Anemic pregnant women had significantly lower serum vitamin D, selenium, zinc, and calcium concentrations and significantly higher copper concentrations than non-anemic women. Among these factors, vitamin D deficiency showed the strongest association with anemia, followed by selenium, zinc, calcium, and copper abnormalities. The findings are broadly consistent with previous research and support the importance of considering multiple micronutrient deficiencies in the assessment of maternal anemia. However, the observed associations should not be interpreted as causal, and prospective studies and randomized intervention trials are needed to determine whether correction of these micronutrient abnormalities can improve maternal hematological outcomes.

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