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Role of Vitamin D Supplementation in Improving Hemoglobin Among Pregnant Women

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Abstract:
Hypovitaminosis D and iron deficiency anemia are prevalent global health concerns, particularly among pregnant women. Both conditions are associated with adverse maternal and fetal outcomes. While vitamin D and iron are essential during pregnancy, the nature of their interaction remains unclear. This study aimed to assess the effect of vitamin D supplementation on hemoglobin (Hb) levels and overall hematological status in pregnant women with mild to moderate iron deficiency anemia. An interventional study was conducted involving 50 pregnant women diagnosed with mild to moderate iron deficiency anemia. Participants received different forms of vitamin D supplementation over an average period of 24.62 ± 8.31 weeks. Serum levels of vitamin D, hemoglobin, and ferritin were measured both before and after treatment. Pearson correlation analysis was used to evaluate associations between vitamin D and hematological markers, with statistical significance set at $p < 0.05$.The mean age of participants was 33.38 ± 5.79 years. Vitamin D levels significantly improved following supplementation, increasing from a deficient baseline of 12.62 ± 5.80 ng/mL to 20.95 ± 7.24 ng/mL. Despite this improvement, no significant correlations were found between pre-treatment vitamin D levels and hemoglobin ($r = 0.045$, $p = 0.757$), or ferritin ($r = 0.199$, $p = 0.165$). Similarly, post-treatment values showed no significant association between vitamin D and ferritin ($r = 0.068$, $p = 0.640$), with only weak correlations observed for hemoglobin. In conclusion, vitamin D supplementation effectively raised vitamin D levels in pregnant women with iron deficiency anemia, but showed no significant correlation with hemoglobin or ferritin levels. These findings suggest a more complex interaction between vitamin D and iron metabolism, highlighting the need for further research, including randomized controlled trials and assessment of other regulatory markers such as hepcidin.

Keywords: Vitamin D supplementation, Hemoglobin levels, Pregnancy, Maternal health

Introduction

The growing recognition of hypovitaminosis D as a significant health issue worldwide has spurred increased research, shifting from observational studies to clinical trials. Hypovitaminosis D, characterized by a serum 25-hydroxy vitamin D (colecalciferol) level below 25 nmol/L, is particularly prevalent in South Asia and the Middle East. Other studies have categorized the condition into deficient, insufficient, and normal levels, depending on the disease in question and the geographical region.(Ahmed, F et al 2021) Pregnancy is associated with a shift toward pro-inflammatory mediators in later stages, while earlier stages typically maintain anti-inflammatory conditions to prevent pregnancy failure. A study by Nicolas established a connection between anemia and inflammation through the gene encoding hepcidin. Subsequent research confirmed that hepcidin plays a crucial role in regulating iron homeostasis, facilitating the transfer of iron stores into the bloodstream. (Ahmed, F et al 2021). relationship between colecalciferol deficiency and anemia among pregnant women in Indonesia had not been previously reported until this article. We hypothesized that there might be an association between colecalciferol and hemoglobin levels. Given that iron is essential for hemoglobin production, it is understood that iron metabolism is ultimately regulated by hepcidin in the hepcidin-ferroportin system. (Ahmed, F et al 2021). The significance of the interaction between vitamin D status and other micronutrients, especially during pregnancy, has been acknowledged. However, there is currently a lack of data exploring the relationship between vitamin D and other micronutrients. (Aiello, G., et al 2024). Iron plays a crucial role in maintaining the body's homeostasis by supporting anemia management and ensuring proper energy production

and transport. The global efforts to address iron deficiency anemia (IDA) have primarily focused on iron supplementation. Recent evidence has suggested a link between iron and vitamin D pathways. Vitamin D deficiency has been shown to contribute to the upregulation of hepcidin, a molecule that reduces iron absorption and traps iron within tissues. Furthermore, vitamin D receptors are found in nearly all cells, including bone marrow progenitor cells. Studies have demonstrated that calcitriol can increase the expression of erythropoietin receptors, thereby stimulating erythropoiesis. (Banasik, C. 2024). While both vitamin D and iron are recognized as crucial for maternal health and fetal development during pregnancy, the understanding of their correlation and regulation throughout pregnancy remains limited. To address this gap, a systematic review and meta-analysis of all available observational studies evaluating the relationship between vitamin D deficiency and anemia in pregnant women was conducted. The goal was to explore the mechanisms of vitamin D and iron regulation during pregnancy and their effects on both the mother and fetus. Additionally, given that vitamin D deficiency and anemia are known risk factors for adverse pregnancy outcomes, this study also aims to investigate the association between vitamin D deficiency and gestational anemia through observational studies. (Basutkar, R 2019). Vitamin D refers to a group of structurally related, fat-soluble compounds that play a key role in enhancing the intestinal absorption of calcium, magnesium, and phosphate, among other vital biological functions. In humans, the two most important forms are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). (Basutkar, R et al 2019). Vitamin D can be obtained either through the diet or produced in the skin upon exposure to ultraviolet B (UVB) radiation, which converts 7-dehydrocholesterol into vitamin D3 (cholecalciferol). Dietary sources include both vitamin D3 and vitamin D2 (ergocalciferol), but few foods naturally contain significant amounts, making dietary intake generally low. This, combined with indoor lifestyles and sun-avoidant behaviors—such as the use of sunscreen—has contributed to widespread vitamin D deficiency. While the effects of deficiency on bone health, including calcium malabsorption and skeletal fragility, are well known, more recent research links low vitamin D levels to muscle weakness, increased risk of falls, and a range of potential non-skeletal health issues. (Christakos, et al 2013). Synthesis and action of vitamin D**:** 7-dehydrocholesterol (7-DHC), a precursor of vitamin D, is part of the Kandutsch-Russell cholesterol pathway. The conversion of 7-DHC to cholesterol is facilitated by the enzyme 7-dehydrocholesterol reductase, which is regulated by factors such as vitamin D and cholesterol. These factors promote the degradation of 7-DHC, leading to increased levels available for vitamin D synthesis. (Cullingford, D et al 2000). The active form of vitamin D, 1,25(OH)2D, acts as a ligand for the vitamin D receptor (VDR), a transcription factor. The effects of 1,25(OH)2D are primarily mediated through vitamin D response elements (VDREs), which influence the expression of genes that contain specific DNA sequences known as VDREs. These elements can be located far from the gene's coding region. Some effects of 1,25(OH)2D occur more rapidly and may involve membrane-bound vitamin D receptors or actions outside the nucleus. Additionally, certain effects do not require the presence of 1,25(OH)2D. Recent advancements have significantly enhanced our understanding of how VDREs regulate gene expression. (Basutkar, R et al 2019)

As illustrated in Figure 1:

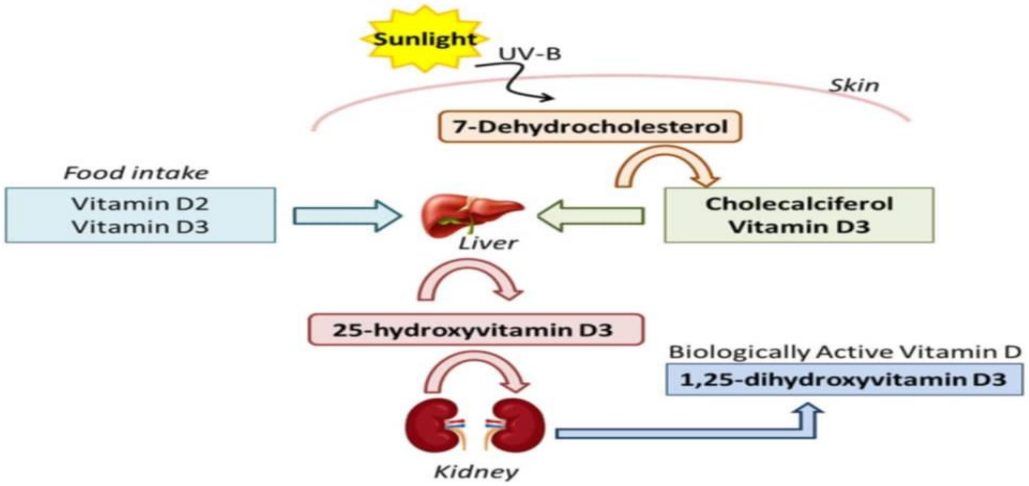

Figure 1: pathway of vitamin d production. (Christakos, et al 2013)

3. Association Between Vitamin D Deficiency and Anemia Risk

Vitamin D deficiency and anemia are significant public health issues, affecting individuals across various populations, including children, elderly adults, individuals with chronic kidney disease (CKD), and those with heart failure . Recent studies have further highlighted this association, showing that low vitamin D levels are inversely linked to the likelihood of anemia in patients scheduled for cardiac surgery and positively correlated with higher hemoglobin levels in elderly men living in the community. New research has also focused on racial and ethnic differences in the vitamin D-anemia relationship, aiming to better understand how vitamin D interacts with different subtypes of anemia.(Judistiani, R et al 2018). Iron is crucial for maintaining proper physiological function, primarily because it is a key component of hemoglobin, which red blood cells (RBCs) use to transport oxygen throughout the body. Additionally, iron plays an essential role in various metabolic processes, serving as a vital cofactor in numerous enzymes. Anemia, a condition marked by reduced oxygen-carrying capacity of the blood, can arise from several causes—iron deficiency being the most common. According to the World Health Organization (2023), anemia affects approximately one-third of the global population, with about half of those cases resulting from iron deficiency. Symptoms of anemia include fatigue, headaches, impaired cognitive function, weakened immunity, and stunted growth in children. It can also be a precursor to more serious health conditions.(Banasik C. 2024). Iron must be obtained from the diet, mainly through animal sources that provide it in the heme or ferric (Fe^{3+}) form, though some plant-based foods also contain iron. Iron homeostasis is tightly regulated by a network of proteins that control its storage and transport into and out of cells (see Figures 2 and 3). It is stored primarily in intestinal cells (enterocytes), liver cells (hepatocytes), and macrophages. In the bloodstream, iron is transported by the protein transferrin and stored in cells by ferritin, which reflects the body's overall iron status. The liver-derived hormone hepcidin helps regulate iron balance by promoting iron storage and inhibiting its release. While iron deficiency and anemia are more widespread concerns, iron overload conditions also occur and can significantly impact health, contributing to both morbidity and mortality.(Banasik C. 2024). Dietary iron is absorbed by enterocytes in the intestine and released into the bloodstream, where it is transported by transferrin. Iron is stored in the liver and utilized for red blood cell production. Iron from aging or damaged red blood cells is recycled by macrophages.(Kiely, M. et al 2001). Vitamin D plays a role in regulating iron metabolism by suppressing hepcidin levels, which increases the availability of iron for red blood cell production and may help mitigate iron deficiency anemia. The pathway from vitamin D precursors to increased serum ferritin is complex and influenced by a network of genes encoding enzymes, proteins, hormones, receptors, transcription factors, and other regulatory molecules. Although elevated hepcidin is typically associated with increased iron recycling, it can also impair iron absorption when chronically high. .(Kiely, M. et al 2001). Iron deficiency and anemia are more common among females—particularly due to menstruation and pregnancy—as well as vegetarians, vegans, certain athletes, and individuals with health conditions such as kidney disease. Inflammatory responses during menstruation can further elevate hepcidin levels, compounding the challenge of maintaining adequate iron status in the presence of blood loss. Vegetarian and vegan diets may lack sufficient vitamin D, iron, and other micronutrients, contributing to lower blood concentrations of both vitamin D and iron.(Kiely, M. et al 2001). **Vitamin D in Hematopoiesis and Hematopoietic Stem Cells** The biologically active form of vitamin D, 1,25-dihydroxycholecalciferol (1,25(OH)2D3), has been shown to promote the monocytic differentiation of HL60, a human promyelocytic leukemia cell line. It is well established that 1,25(OH)2D3 can induce normal mononuclear blood cells to differentiate along the monocyte-macrophage maturation pathway. In vitro studies have demonstrated that vitamin D suppresses colony formation of normal human granulocyte-macrophage progenitors (CFU-GM), while promoting the differentiation of these colonies into monocyte-macrophages. Experiments using hematopoietic stem cells and leukemic cell lines treated with the active form of vitamin D show an increase in monocyte/macrophage differentiation and a higher number of mature cells, effects that are absent in mice lacking the vitamin D receptor (VDR) . Upon binding to the VDR, vitamin D triggers these differentiation processes. (Cullingford, D et al 2000)

Material and methods

A cross-sectional study design will be employed to assess vitamin D levels and hemoglobin concentrations among pregnant participants at a single healthcare facility. This design allows for the simultaneous measurement of both variables.

Sample

The target population consists of pregnant women aged 18–45 attending the antenatal clinic. A sample of 50 participants will be selected through convenience sampling. Inclusion criteria include pregnant women who consent to participate and have no chronic illnesses affecting vitamin D or hemoglobin levels (e.g., renal disease, hematologic disorders).

Data Collection Tools

Data will be collected using the following tools:

- Serum Vitamin D Levels: Measured using a standardized immunoassay.
- Anemia Assessment: Hemoglobin levels will be determined using a complete blood count (CBC).
- Demographic and Clinical Data: Information such as maternal age, gestational age, gravidity, dietary habits, and sun exposure will be collected through a structured questionnaire.

Data Collection Procedures

Participants will be recruited during their routine antenatal visits. Blood samples will be drawn to measure serum vitamin D and hemoglobin levels. A structured questionnaire will be administered to gather demographic and lifestyle data.

Data Analysis

Data will be analyzed using statistical software (e.g., SPSS). Pearson correlation coefficients will be calculated to assess the relationship between vitamin D levels and hemoglobin concentrations. A p-value of <0.05 will be considered statistically significant.

Ethical Considerations

Ethical approval will be obtained from the Institutional Review Board (IRB). Informed consent will be obtained from all participants, ensuring they are aware of the study's purpose and their right to withdraw at any time without affecting their medical care.

Results and Discussion

A total of 50 pregnant women participated in this study. The mean age of the pregnant women was 33.38 ± 5.79 years, with ages ranging from 22 to 45 years. Half of the pregnant women (25 participants, 50%) were under 30 years old, 17 (34%) were between 31 and 40 years, and 8 (16%) were over 40 years. The mean duration of treatment was 24.62 ± 8.31 weeks, with a range from 11 to 42 weeks (Table 1, Figure 4).

Table 1: Age of pregnant women

	No	Percentage %
<30	**17**	**34.0**
31-40	**25**	**50.0**
>40	**8**	**16.0**

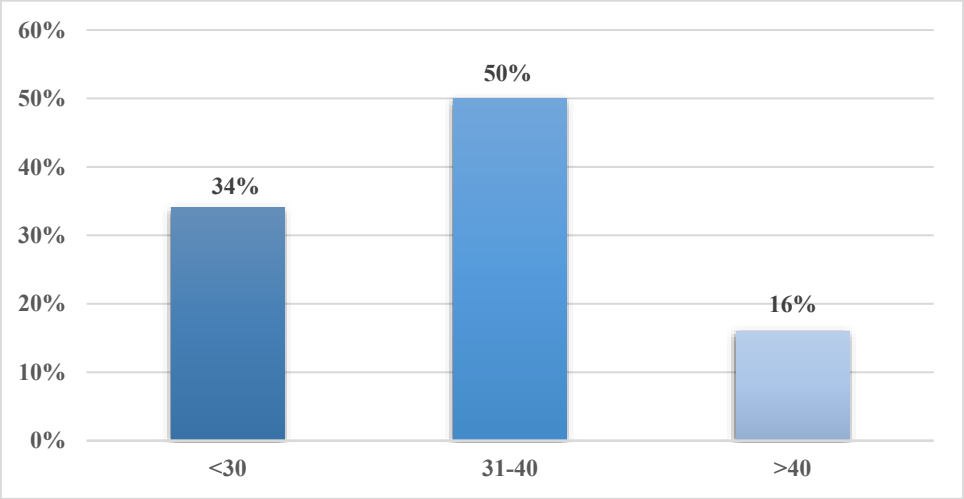

Figure 4: Age of pregnant women

Table 2: The different types of Vitamin D supplements used by the pregnant women

	No	Percentage %
3m 50,000	1	2.0
Cap forte 50,000	2	4.0
inj 200,000	14	28.0
inj 50,000	15	30.0
D-cure	16	32.0
Cap 50,000	17	34.0

This table shows the different types of Vitamin D supplements used by the pregnant women in the study. The most commonly used form was Cap 50,000 IU, taken by 34% (17 women), followed by D-cure, used by 32% (16 women). Injection 50,000 IU and Injection 200,000 IU were used by 30% (15 women) and 28% (14 women), respectively. Less commonly used were Cap forte 50,000 IU (4%) and 3m 50,000 IU (2%) (Table 2).

Table 3: The Vitamin D levels of pregnant women before and post receiving supplementation

	Minimum	Maximum	Mean	St. Deviation
Before vit D	4.0	26.0	12.618	5.7968
After vit D	8.00	36.00	20.946	7.2405

Before treatment, Vitamin D levels ranged from 4.0 to 26.0 ng/mL, with a mean of 12.62 ± 5.80 ng/mL, indicating deficiency in many cases. Post treatment, levels increased significantly, ranging from 8.0 to 36.0 ng/mL, with a higher mean of 20.95 ± 7.24 ng/mL. These findings demonstrate a positive response to Vitamin D supplementation and suggest improved Vitamin D status among the participants (Table 3).

Table 4: Correlation between before-treatment Hb and before-treatment Vitamin D levels

	mean±SD	R	P- Value
Hb	8.94 ± 1.03	0.045	0.757
Vitamin D	12.62 ± 5.80		

A Pearson correlation analysis was conducted to examine the relationship between before -treatment Vitamin D levels and before -treatment hemoglobin (Hb) levels. The analysis revealed a weak positive correlation (r = 0.045) with a p-value of 0.757. Since the p-value is greater than 0.05, the correlation is not statistically significant (Table 4).

Table 5: Correlation between before-treatment Hb and before-treatment Vitamin D levels

	Mean±SD	R	P value
Ferritin level	8.35 ± 2.52	0.199	0.165
Vitamin D	12.62 ± 5.80		

A Pearson correlation analysis was conducted to examine the relationship between before -treatment Vitamin D levels and Ferritin level. The analysis revealed a weak positive correlation (r = 0.199) with a p-value of 0.165Since the p-value is greater than 0.05, the correlation is not statistically significant (Table 5).

Table 6: Correlation between post-treatment ferritin and post-treatment Vitamin D levels

		R	P- Value
Ferritin level	34.33 ± 8.10	0.068	0.640
Vitamin D	20.95 ± 7.24		

A Pearson correlation analysis was conducted to assess the relationship between post-treatment ferritin and post-treatment Vitamin D levels. The results showed a very weak positive correlation (r = 0.068) with a p-value of 0.640. Since the p-value is greater than 0.05, the correlation is not statistically significant (Table 6).

Scatter plot for vitamin D compared to parameters

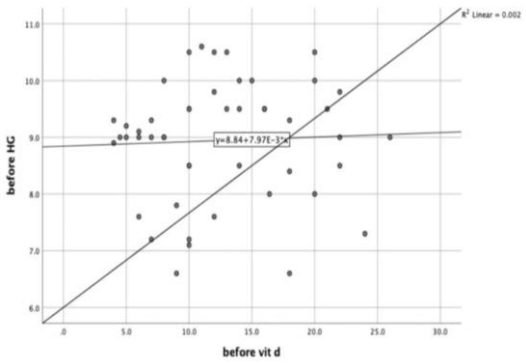

Figure 5.a

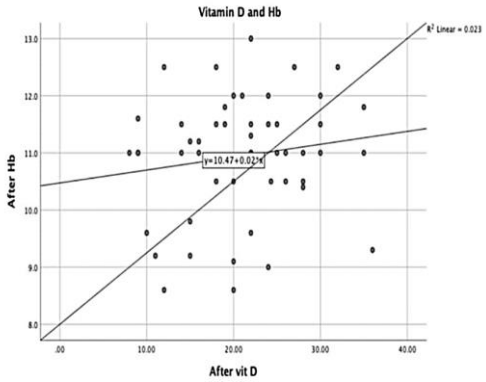

Figure 5.b

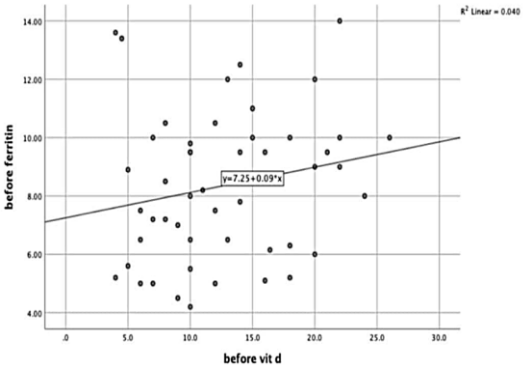

Figure 5.c

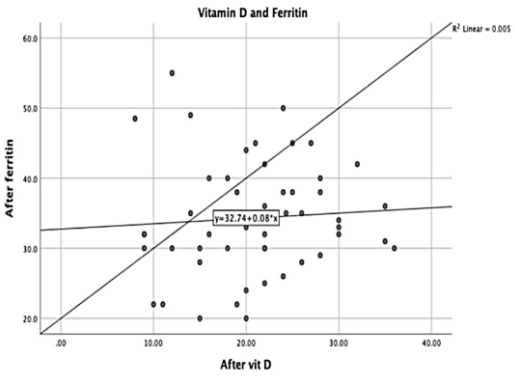

Figure 5.d

5.a: Scatter plot for vitamin D compared to HG levels before treatment. 5.b: Scatter plot for vitamin D compared to HG levels after treatment. 5.c: Scatter plot for vitamin D compared to ferritin levels before treatment. 5.d: Scatter plot for vitamin D compared to ferritin levels after treatment.

The scatter plot illustrates the relationship between before-treatment Vitamin D levels and before-treatment ferritin levels among the participants. This very low r value indicates that there is no significant linear correlation between Vitamin D and Hb levels before treatment (Figure 5.a). The scatter plot illustrates the relationship between post-treatment Vitamin D levels and post-treatment Hb among the participants. This very low r value indicates that there is no significant linear correlation between Vitamin D and Hb after treatment (Figure 5.b). The scatter plot illustrates the relationship between before -treatment Vitamin D levels and before t-treatment ferritin levels among the participants. This very low r value indicates that there is no significant linear correlation between Vitamin D and ferritin levels before treatment (Figure 5.c). The scatter plot illustrates the relationship between post-treatment Vitamin D levels and before-treatment ferritin levels among the participants. This very low r value indicates that there is no significant linear correlation between Vitamin D and ferritin levels before treatment (Figure 5.d). This study aimed to evaluate the effect of vitamin D supplementation on hemoglobin (Hb) levels and overall hematological health in pregnant women, specifically investigating whether adequate vitamin D levels could improve Hb concentrations and reduce the risk of anemia during pregnancy. Our findings, based on a cohort of 50 pregnant women, provide insights into the dynamics of vitamin D status and its relationship with iron parameters before and after supplementation. The demographic data revealed a diverse age range among participants, with a mean age of 33.38 ± 5.79 years, reflecting a representative sample of pregnant women. The varied treatment durations further highlight the real-world application of the study. The significant increase in mean vitamin D levels from 12.62 ± 5.80 ng/mL before treatment to 20.95 ± 7.24 ng/mL after supplementation clearly demonstrates the effectiveness of vitamin D supplementation in improving vitamin D status among pregnant women with initially deficient or insufficient levels. This positive response aligns with the well-established efficacy of vitamin D interventions in correcting hypovitaminosis D. The variety of vitamin D supplements used, with "Cap 50,000 IU" and "D-cure" being the most common, suggests common clinical practices in managing vitamin D deficiency.

Despite the successful elevation of vitamin D levels, our core hypothesis regarding a direct association between vitamin D and hemoglobin or ferritin levels was not statistically supported by the data. Both pre-treatment and post-treatment analyses showed weak, non-statistically significant correlations between vitamin D levels and Hb levels ($r = 0.045$, $p = 0.757$ before treatment; r values not explicitly provided for post-treatment Hb but indicated as "very low r value" in scatter plots), as well as between vitamin D levels and ferritin levels **($r = 0.199$, $p =$** 0.165 before treatment; $r = 0.068$, $p = 0.640$ after treatment). The scatter plots visually reinforced this lack of a significant linear relationship. These findings contrast with some of the existing literature cited in the introduction, which suggested a potential link between vitamin D and iron metabolism. For instance, studies by Suh YJ et al. and Lee JA et al. indicated a negative association between vitamin D levels and iron deficiency anemia, while Qader EA et al. found a positive correlation between vitamin D and serum iron in children. The discrepancy in our findings could be attributed to several factors. Firstly, the sample size of 50 pregnant women might be insufficient to detect subtle correlations, especially if the true effect size is small. Larger studies might be needed to identify such relationships. Secondly, the severity of anemia in our study, described as "mild to moderate iron deficiency anemia" in the introduction's aims, might influence the observed correlation. In cases of more severe deficiency, the interaction between vitamin D and iron pathways might be more pronounced.
Furthermore, the complex interplay of factors influencing both vitamin D and iron status during pregnancy needs to be considered. Pregnancy itself is associated with physiological changes that impact iron homeostasis, including increased demand for iron due to expanding blood volume and fetal development. While the introduction highlights the role of hepcidin in iron regulation and its potential upregulation by vitamin D deficiency, our study did not directly measure hepcidin levels. Future research could explore the direct impact of vitamin D supplementation on hepcidin and other iron regulatory proteins to elucidate the underlying mechanisms. The duration of treatment and the specific dosages and forms of vitamin D supplementation might also play a role. While our study documented the types of supplements, a more granular analysis of dosage and patient adherence could provide further insights. It is also important to acknowledge that this study focused solely on iron and hemoglobin, while vitamin D interacts with a broader range of micronutrients crucial for maternal and fetal health, as noted in the introduction. Limitations and Future Directions
The study has several limitations. The relatively small sample size might limit the generalizability of our findings and the power to detect statistically significant correlations. We did not control for other potential confounders that could influence hemoglobin or ferritin levels, such as dietary iron intake, parasitic infections, or genetic predispositions to anemia. Additionally, the study did not follow participants for long-term outcomes, such as pregnancy complications or neonatal health, which are often linked to both vitamin D deficiency and anemia.
Future research should consider:

- Larger sample sizes to increase statistical power.
- Prospective, randomized controlled trials to establish causality between vitamin D supplementation and hematological parameters.
- Measurement of hepcidin levels and other iron regulatory markers to understand the mechanistic link, if any.
- Assessment of dietary intake and other lifestyle factors that could influence both vitamin D and iron status.
- Longitudinal studies to observe the long-term impact of vitamin D supplementation on maternal and fetal health outcomes in anemic pregnant women.

Conclusion

. This study successfully demonstrated that vitamin D supplementation effectively raises vitamin D levels in pregnant women. However, it did not find a statistically significant correlation between vitamin D levels and either hemoglobin or ferritin levels before or after supplementation in our cohort of pregnant women with mild to moderate iron deficiency anemia. These findings suggest that while vitamin D supplementation is crucial for addressing hypovitaminosis D in pregnant women, its direct impact on hemoglobin and ferritin levels may not be as straightforward as hypothesized, or it may require a larger sample size and more targeted investigation into the underlying regulatory pathways. Further research is warranted to fully understand the intricate relationship between vitamin D and iron metabolism during pregnancy.

References

Ahmed, F., Khosravi-Boroujeni, H., Khan, M. R., Roy, A. K., & Raqib, R. (2021). Prevalence and predictors of vitamin D deficiency and insufficiency among pregnant rural women in Bangladesh. *Nutrients*, *13*(2), 449.

Aiello, G., Lombardo, M., & Baldelli, S. (2024). Exploring vitamin D synthesis and function in cardiovascular health: A narrative review. *Applied Sciences*, *14*(11), 4339.

Banasik, C. (2024). *Associations of polymorphisms in vitamin D and iron-related genes with vitamin D supplementation and iron status in women* (Doctoral dissertation).

Basutkar, R. S., Eipe, T., Tsundue, T., Perumal, D., & Ponnusankar, S. (2019). Reduced vitamin D levels and iron deficiency anaemia in pregnant women: an evolving correlation. *Journal of Young Pharmacists*, *11*(1), 92.

Bookshelf, N. C. B. I. (2000). A service of the National Library of Medicine. *National Institutes of Health*.

Christakos, S., Hewison, M., Gardner, D. G., Wagner, C. L., Sergeev, I. N., Rutten, E., ... & Bikle, D. D. (2013). Vitamin D: beyond bone. *Annals of the New York Academy of Sciences*, *1287*(1), 45-58.

Cullingford, D. J., Siafarikas, A., & Choong, C. S. (2000). A service of the National library of medicine. National Institutes of Health. Feingold KR. MDText. com.

Judistiani, R. T. D., Gumilang, L., Nirmala, S. A., Irianti, S., Wirhana, D., Permana, I., ... & Setiabudiawan, B. (2018). Association of colecalciferol, ferritin, and anemia among pregnant women: result from cohort study on vitamin D status and its impact during pregnancy and childhood in Indonesia. *Anemia*, *2018*(1), 2047981.

Kiely, M. E., McCarthy, E. K., & Hennessy, Á. (2021). Iron, iodine and vitamin D deficiencies during pregnancy: epidemiology, risk factors and developmental impacts. *Proceedings of the Nutrition Society*, *80*(3), 290-302.