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The Hidden Link: Vitamin D Deficiency and Its Association with Anemia and Red Blood Cell Health in Pregnancy

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ABSTRACT

Background: Vitamin D deficiency and anemia are common comorbidities in pregnancy, both independently linked with adverse maternal and fetal outcomes. However, the interrelationship between maternal vitamin D status and hematological health, particularly red blood cell indices, remains incompletely understood.

Objective: This study aimed to investigate the association between maternal vitamin D deficiency and anemia, with a specific focus on evaluating the impact of vitamin D status on red cell parameters among pregnant women.

Methods: A cross-sectional analysis was conducted involving 150 anemic and 150 non-anemic pregnant women. Serum vitamin D and comprehensive hematological indices—including hemoglobin, red blood cell (RBC) count, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet count—were measured and compared between groups. Correlation analyses explored the relationship between vitamin D and red cell indices.

Results: Anemic women exhibited significantly lower mean vitamin D levels (9.6 ± 5.0 ng/ml) compared to non-anemic women (29.4 ± 6.0 ng/ml; $p < 0.001$). All major hematological indices (hemoglobin, RBC, hematocrit, MCV, MCH, MCHC) were reduced, while RDW was elevated in the anemic group (all $p < 0.001$). Vitamin D levels positively correlated with hemoglobin ($r = 0.65$), RBC ($r = 0.62$), and red cell indices, and inversely with RDW ($r = –0.51$; each $p < 0.05$).

Conclusion: Vitamin D deficiency is strongly associated with anemia and impaired red cell health in pregnancy. Integrating vitamin D screening and supplementation into antenatal care may reduce the burden of gestational anemia and improve maternal outcomes.

Keywords: *Vitamin D deficiency, anemia, pregnancy, red blood cell indices, maternal health*

1. INTRODUCTION

Vitamin D deficiency is a widespread public health concern, particularly among pregnant women, with prevalence rates ranging from 5% to as high as 50% worldwide [1-3]. During pregnancy, adequate vitamin D status is crucial for maternal health, fetal skeletal development, and optimal gestational outcomes [4,5]. The increased calcium demands in the third trimester further underscore the importance of maintaining sufficient vitamin D levels [6]. Beyond its classical role in calcium homeostasis and bone metabolism, emerging evidence suggests that vitamin D plays a broader role in haematopoiesis and immune modulation [7,8].

Anemia in pregnancy remains a significant cause of maternal and fetal morbidity, with altered red blood cell indices being central to its diagnosis and management [9]. While iron deficiency is the most common cause of gestational anaemia, recent

studies highlight a potential link between vitamin D deficiency and anaemia, suggesting vitamin D's involvement in erythropoiesis and iron metabolism [10-12]. Observational studies and meta-analyses indicate that vitamin D deficiency may increase the risk of anaemia during pregnancy, possibly through mechanisms involving inflammation, altered iron homeostasis, and bone marrow function [7,9,11].

This study titled, aims to elucidate the relationship between maternal vitamin D levels and haematological parameters in pregnant women. By comparing anemic and non-anaemic cohorts with respect to their vitamin D status and detailed red blood cell indices, this work seeks to explore how vitamin D deficiency may contribute to the pathophysiology of gestational anaemia and its haematological manifestations. Understanding these associations could inform improved screening, prevention, and therapeutic strategies to optimize maternal and fetal health outcomes.

This investigation builds upon recent findings that report 61% increased odds of anaemia in vitamin D deficient pregnant women and underscores the necessity of considering vitamin D as more than just a skeletal health nutrient during pregnancy. As per the above content, the aim of this study is to investigate the association between maternal vitamin D deficiency and anaemia during pregnancy, with a focus on evaluating the impact of vitamin D status on red blood cell health and haematological indices

2. METHODOLOGY

Study Population

The cross-sectional study was carried out at maternity centre, Gonda Medical centre, Gonda and the experimental work was done in the Department of Biochemistry, Dr. Ram Manohar lohia institute of Medical Sciences, Lucknow. Total 300 pregnant women was recruited in this study. Based on the hemoglobin status women were divided into two groups: anaemic pregnant women (n=150) and non-anaemic pregnant women (n=150). The classification of anemia followed the WHO guidelines, categorizing the women into Mild anemia (Hb: 9-11g/dl), Moderate anemia (Hb: 7-9g/dl), and Severe anemia (Hb: <7g/dl) [13].

Ethic

The study was approved by the Institutional Ethical Committee (IEC) of NIMS University, Rajasthan, Jaipur (Ref. NIMSUR/URC/2024/400). It adhered to ethical guidelines as per the Helsinki Declaration. Informed consent was obtained from all participants, ensuring they understood the study's purpose, procedures, and potential risks. Participants were assured of their right to withdraw at any time without consequence, and their confidentiality was strictly maintained throughout the study.

Sample collection and processing

Sample was collected during the first visit to the maternity centre at Gonda Medical centre Blood sample (5mL) was collected by veinpuncture: 2 mL in EDTA vial and 3 mL in a plain vial for serum. Serum sample was collected after centrifugation at 3500 rpm for 15 minutes. EDTA sample was used to analysed the CBC while, serum sample was used to analyse the essential minerals.

Estimation of complete blood count (CBC)

The complete blood count (CBC) was measured using a fully automated 5-part differential hematology analyzer, which provided detailed counts of red blood cells, red cell indices, haemoglobin concentration, platelet count.

Estimation of Vitamin D using Liquid chromatography tandem mass spectrometry (LCMS/MS).

A 500µL serum sample was used to measure vitamins A using LC-MS/MS. First, Protein precipitation was done using a 0.125 mL of 0.1 M trichloroacetic acid concentration. The supernatant was separated, and fat-soluble vitamins were extracted using 1 mL hexane and centrifugated, then evaporated using a nitrogen evaporator. The residue was reconstituted in 100 µL of mobile phase. The residue was reconstituted in 100 µL of mobile phase, consisting of water (phase 1) and methanol (phase 2), modified with 0.5 mM ammonium formate. The mixture was sonicated for 30 seconds to ensure complete dissolution. The final solution was transferred to an autosampler vial for injection into the LC-MS/MS system.

Instrumental Conditions

The analysis used an AB Sciex UPLC system with a triple quadrupole 5500+ MS/MS (Sciex, Framingham, MA, USA). The ionization interface was operated in positive ion mode with the following settings: curtain gas at 50 PSI, collision-activated dissociation gas at 10 psi, a temperature of 450°C, nebulizer gas at 50 psi, and turbo ion spray voltage at 4500 V. Chromatographic separation was performed using a C18 2.5-µm analytical column (Kinetex, 2.1 × 50 mm) maintained at 40°C. The residue was reconstituted in 100 µL of mobile phase, consisting of water (phase 1) and methanol (phase 2), modified with 0.5 mM ammonium formate. The mixture was sonicated for 30 seconds to ensure complete dissolution. Serial dilutions were made to establish a calibration curve with levels ranging from 0.0, 0.5, 1, 2, 5, and 10 ng/L (Fig.1). Quality control was maintained by analysis of standard reference materials (National Institute of Standards and Technology, NIST,

Gaithersburg, MD, USA).

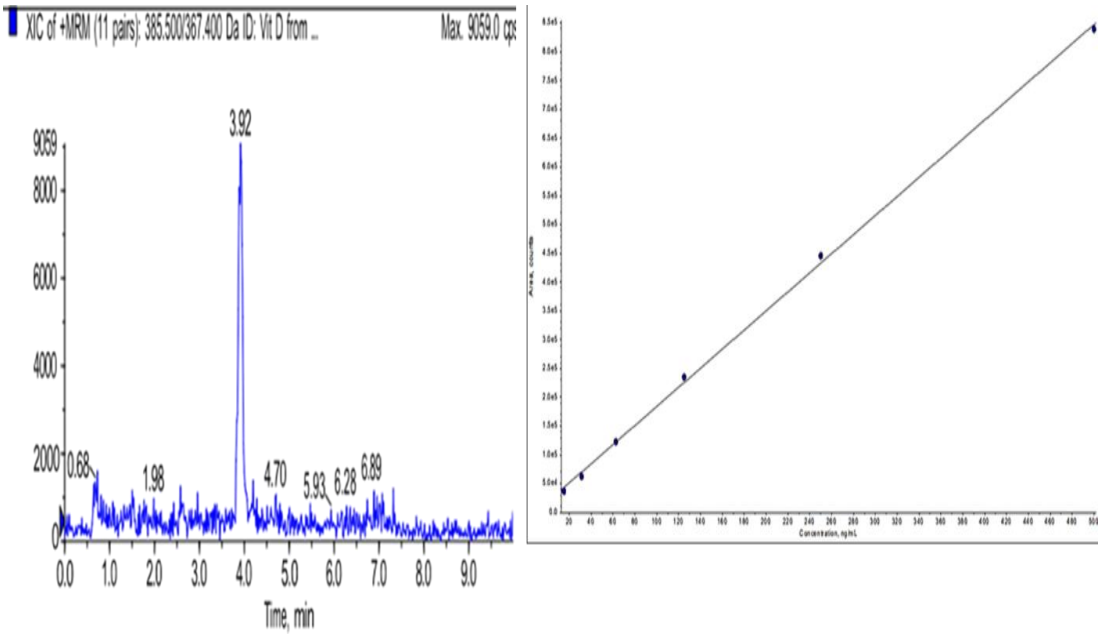


Fig. 1: Chromatogram and Calibration curve of Vitamin D on LCMS/MS

Data analysis

Data were presented as mean and standard deviation for continuous variables. The Student's t-test was used to assess statistical differences between anaemic and non-anaemic pregnant women. Pearson correlation analysis was performed to examine the relationships between variables. Data were analysed using SPSS software version 24 (IBM Corp., Chicago, USA), and graphs were prepared using Python software. A p-value of <0.05 was considered statistically significant.

Result

Status of vitamin D and red cell indices'

The results showed that pregnant women with anemia had significantly lower mean vitamin D levels (9.6 ± 5.0 ng/ml) compared to non-anemic women (29.4 ± 6.0 ng/ml), with a p-value < 0.001. Hemoglobin concentration was also significantly reduced in the anemic group (9.5 ± 0.8 g/dL vs. 12.5 ± 0.7 g/dL, p < 0.001), along with lower RBC count, hematocrit, MCV, MCH, and MCHC values (all p < 0.001). Additionally, RDW was elevated (15.2 ± 1.5% vs. 13.1 ± 1.2%, p < 0.001), reflecting greater red cell size variability in anemia. Platelet counts were also significantly decreased in anemic pregnant women (1.8 ± 0.3 vs. 2.6 ± 0.4 x10^5/µL, p < 0.001). These findings collectively demonstrate a strong association between vitamin D deficiency and impaired hematological indices indicative of anemia in pregnancy, underscoring the importance of monitoring and managing vitamin D levels alongside traditional hematological parameters to improve maternal and fetal health outcomes.

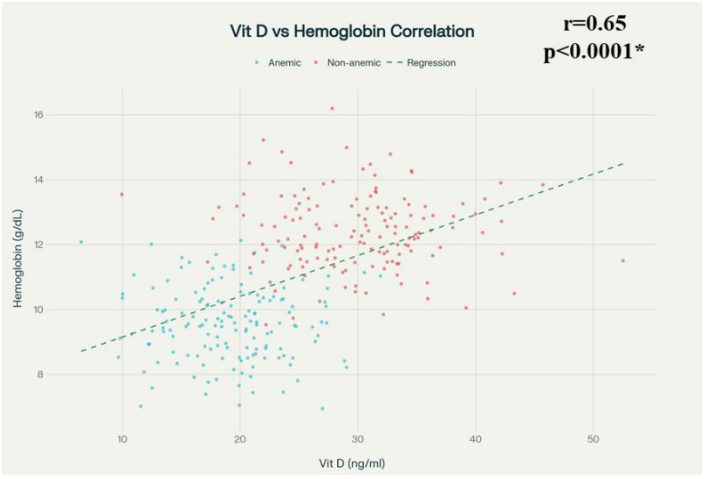
Table 1: Comparison of vitamin D and haematogical parameters in anaemic and non-anaemic pregnant women

Parameter	Anaemic (n=150) (Mean ± SD)	Non-anaemic (n=150) (Mean ± SD)	p-value
Vitamin D (ng/ml)	9.6 ± 5.0	29.4 ± 6.0	<0.001*
Hemoglobin (g/dL)	9.5 ± 0.8	12.5 ± 0.7	<0.001*
RBC (million/µL)	3.8 ± 0.6	4.6 ± 0.5	<0.001*
Hematocrit (%)	29 ± 4	36 ± 4	<0.001*
MCV (fL)	72 ± 5	83 ± 6	<0.001*
MCH (pg)	23 ± 2	29 ± 3	<0.001*
MCHC (g/dL)	30 ± 2	34 ± 2	<0.001*
RDW (%)	15.2 ± 1.5	13.1 ± 1.2	<0.001*
Platelets (x10^5/µL)	1.8 ± 0.3	2.6 ± 0.4	<0.001*

*Abbreviations: MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell hemoglobin concentration, RDW: Red distribution width. *p<0.05 was considered as statistically significant.*

Association of Hemoglobin and RBC with vitamin D

Analysis of the study cohort revealed a strong positive correlation between vitamin D levels and key hematological parameters in pregnant women. Specifically, vitamin D concentration was significantly associated with hemoglobin levels ($r = 0.65$, $p < 0.0001$), as depicted by the regression line in the correlation graph. Similarly, red blood cell (RBC) count correlated positively with vitamin D status ($r = 0.62$, $p < 0.0001$), indicating that women with higher vitamin D levels tended to maintain healthier hemoglobin and RBC counts.



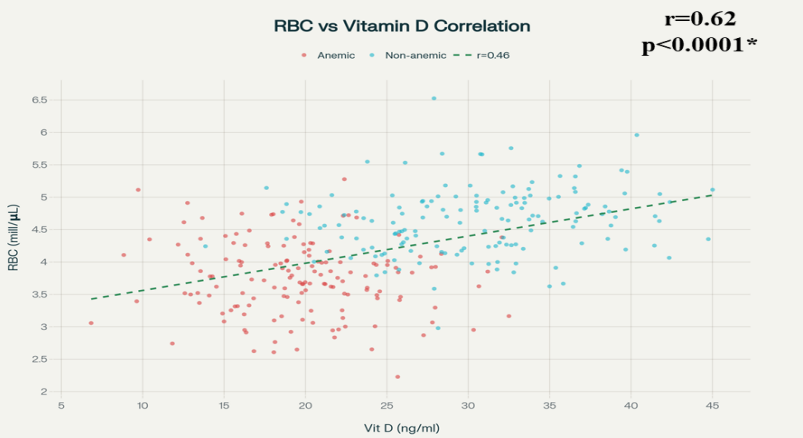


Fig.2: Correlation of Hemoglobin, red cell indices with vitamin D.

Distinct separation between anemic (shown in blue) and non-anemic (shown in red) subjects was observed across both scatter plots, further emphasizing the impact of vitamin D deficiency on anemia-related indices. These findings underscore the hidden link between vitamin D deficiency and impaired red blood cell health, supporting its contributory role in the pathophysiology of gestational anemia.

Correlation of vitamin D with red cell indices

Significant differences were observed in blood parameters between anemic and non-anemic pregnant women. Anemic subjects exhibited markedly lower mean values for key hematological indices including hemoglobin, RBC count, MCV, MCH, and MCHC, along with higher RDW, compared to the non-anemic group (all $p < 0.001$). The mean vitamin D level was substantially lower among anemic women (9.6 ± 5.0 ng/ml) than in non-anemic controls (29.4 ± 6.0 ng/ml, $p < 0.001$), confirming the co-occurrence of vitamin D deficiency and anemia.

Correlation analyses further highlighted the association between vitamin D status and red blood cell health. Vitamin D showed robust positive correlations with MCV ($r = 0.45$, $p = 0.011$), MCH ($r = 0.50$, $p = 0.006$), and MCHC ($r = 0.55$, $p = 0.001$); in contrast, vitamin D was inversely correlated with RDW ($r = -0.51$, $p = 0.012$), indicating that lower vitamin D relates to greater red cell size variability. Distribution plots illustrated the distinct separation in red cell indices between anemic and non-anemic groups and emphasized that higher vitamin D levels are consistently linked to more favorable hematological profiles.

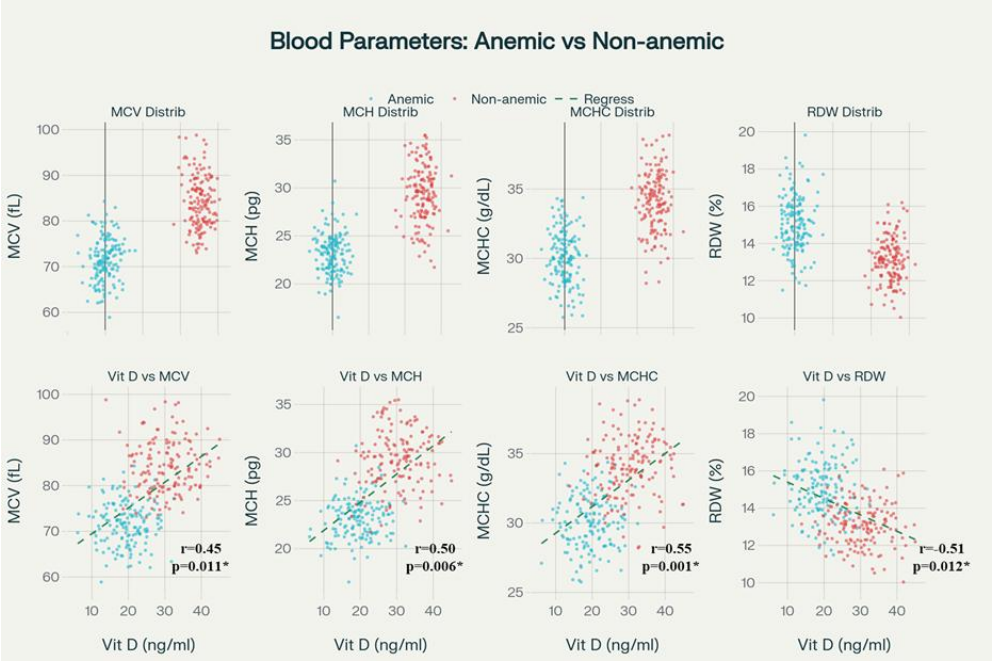


Fig. 3: Correlation and comparison of red cell indices with vitamin D

*Scatter plots and distribution graphs illustrate the relationship between vitamin D levels and red blood cell indices in anemic (blue dots) and non-anemic (red dots) pregnant women. The top row shows groupwise distributions for MCV (fL), MCH (pg), MCHC (g/dL), and RDW (%), highlighting distinct separation between anemic and non-anemic cohorts. The bottom row presents correlation graphs between vitamin D (ng/ml) and each red cell parameter, with regression lines indicating the direction and magnitude of association. *p<0.05 was considered as statistically significant.*

3. DISCUSSION

This study provides compelling evidence for the intricate relationship between maternal vitamin D deficiency and anemia during pregnancy, as established by lower biochemical and hematological indices observed in the anemic group compared to their non-anemic counterparts. The marked reduction in vitamin D levels among anemic pregnant women—averaging 9.6 ± 5.0 ng/ml, versus 29.4 ± 6.0 ng/ml in those without anemia—correlates with significantly impaired red cell parameters, including reduced hemoglobin, RBC count, hematocrit, MCV, MCH, and MCHC, and elevated RDW. These findings support the hypothesis that vitamin D deficiency can adversely affect erythropoiesis and red cell morphology, which is consistent with a rising number of international studies identifying vitamin D insufficiency as an under-recognized contributor to gestational anemia and poor pregnancy outcomes [3,5,6,9,14-16]. The biological rationale for this association stems from vitamin D's pleiotropic effects on hematopoietic progenitors, regulation of iron homeostasis via hepcidin modulation, and anti-inflammatory actions that influence bone marrow function and red cell turnover.

A focal strength of this investigation lies in its robust cohort size and comprehensive approach, presenting clear differences in hematological health between anemic and non-anemic women with appropriate statistical validation. By coupling mean groupwise analysis with scatter plot correlations, the data provide both population-level and individual-level perspectives. Positive correlations between vitamin D and indices such as MCV, MCH, MCHC ($r \sim 0.45–0.55$), as well as Hb and RBC count ($r \sim 0.62–0.65$), reinforce that higher vitamin D status corresponds to healthier, more physiologically optimal red cell characteristics. In addition, the inverse association found between vitamin D and RDW ($r = -0.51$) indicates that severe deficiency is linked with greater variation in red cell size—a hallmark of ineffective erythropoiesis and a predictor of iron-deficiency or mixed nutritional anemia. The detection of such associations, with statistical significance across nearly all parameters, aligns with meta-analyses and clinical reviews demonstrating increased risk of anemia, poor immune modulation, and adverse obstetric outcomes—such as preterm birth, low birth weight, and gestational diabetes—in populations with low vitamin D.

Clinically, these results underscore a necessary paradigm shift from focusing solely on iron supplementation for anemia management during pregnancy to a broader consideration of micronutrient status, particularly vitamin D. Existing data indicate that vitamin D supplementation, either alone or in combination with iron therapy, may improve hemoglobin concentrations and reduce anemia prevalence, especially in those with severe deficiency [1,17-20]. Routine screening for vitamin D status in pregnant women—particularly those diagnosed with anemia—could be integrated into antenatal care pathways, allowing for tailored nutritional interventions and potentially improved maternal/fetal outcomes. Given the biological interplay observed here, public health strategies should emphasize both sufficient sun exposure and dietary intake of vitamin D as part of prenatal education, especially in regions or populations where deficiency is prevalent due to cultural, geographic, or socioeconomic factors [21-23]. Furthermore, the role of vitamin D in modulating hepcidin and thus iron absorption and utilization provides a mechanistic explanation for its dual effect on both bone and blood health—an emerging area of interest for obstetricians and hematologists alike [24-26].

Despite its strengths, this study is subject to several important limitations. The cross-sectional design precludes direct causal inferences, and as such, the directionality of the relationship between vitamin D status and anemia cannot be definitively established. Selection bias may arise from the single-center recruitment and specific demographic characteristics of the study population, limiting generalizability to broader or more diverse populations. Potential confounding variables—including dietary patterns, sunlight exposure, genetic predisposition, coexisting micronutrient deficiencies (such as B vitamins and folate), and unmeasured inflammatory conditions—may also influence the observed associations. Future research should prioritize longitudinal cohort studies and randomized controlled trials to evaluate the impact of vitamin D supplementation, both as a preventive and therapeutic strategy for gestational anemia. Additionally, mechanistic studies exploring vitamin D's effect on erythropoietic pathways, iron mobilization, and immune modulation could further clarify causality and optimize therapeutic recommendations.

The strength of this study lies in its thorough characterization of hematological parameters and robust analysis of the vitamin D–anemia link in a clinically relevant cohort. However, the cross-sectional design and single-center sample limit causal inference and wide generalizability. Ultimately, this work advocates for the integration of vitamin D screening and supplementation within antenatal care, especially for high-risk pregnant populations, and urges future longitudinal and interventional studies to validate and expand these findings. By prioritizing vitamin D sufficiency, clinicians and public health practitioners may significantly improve maternal hematological outcomes and reduce the burden of anemia in pregnancy.

4. CONCLUSION

The present findings serve to spotlight vitamin D deficiency as a hidden yet critical factor in the development and severity of anemia during pregnancy, with significant ramifications for red blood cell health and maternal/fetal well-being. This research adds to a growing consensus that addressing vitamin D status is integral—not supplementary—to effective antenatal care. Targeted screening and appropriate supplementation, in conjunction with iron therapy, may enhance hematological outcomes and reduce pregnancy-related complications. Broadening future research to include global, multi-center data, interventional trials, and mechanistic laboratory analysis will be vital in establishing evidence-based protocols aimed at reducing the burden of anemia and improving maternal health worldwide.

CRediT authorship contribution statement

CM: Sample collection, experiment work, R: Formal analysis, SS: Investigations, Manuscript writing and review, Data analysis, JV: Investigations, RK: Clinical investigation and sampling, MRK: Conceptualization, fund acquisition, manuscript review and editing

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