

## EDUCATIONAL ATTAINMENT MODIFIES THE ASSOCIATION BETWEEN SERUM VITAMIN D AND HEMOGLOBIN CONCENTRATIONS IN WOMEN OF REPRODUCTIVE AGE: A CROSS-SECTIONAL STUDY

Muhammad Imran<sup>1</sup>

<sup>1</sup>Professional College of Medical Sciences, Peshawar

Muhammad Alyian<sup>2</sup>

<sup>2</sup>Professional College of Medical Sciences, Peshawar

Muhammad Atif<sup>3</sup>

<sup>3</sup>Professional College of Medical Sciences, Peshawar

Aqib Marwat<sup>4</sup>

<sup>4</sup>Institute of Paramedical Sciences, Khyber Medical University, Peshawar

Saeed Anwar<sup>5</sup>

<sup>5</sup>Institute of Paramedical Sciences, Khyber Medical University, Peshawar

Abdul Basit<sup>6</sup>

<sup>6</sup>Institute of Paramedical Sciences, Khyber Medical University, Peshawar

Muhammad Asif Zeb<sup>\*7</sup>

<sup>\*7</sup>Institute of Paramedical Sciences, Khyber Medical University, Peshawar

<sup>\*7</sup>[muhammadasif.ipms@kmu.edu.pk](mailto:muhammadasif.ipms@kmu.edu.pk)

### Author Details

#### Keywords:

Vitamin D, Anemia, Educational Status, Women's Health

Received on 18 October 2025

Accepted on 30 November 2025

Published on 18 December 2025

Corresponding E-mails & Authors\*:

Muhammad Asif Zeb<sup>\*7</sup>

<sup>\*7</sup>Institute of Paramedical Sciences, Khyber Medical University, Peshawar

<sup>\*7</sup>[muhammadasif.ipms@kmu.edu.pk](mailto:muhammadasif.ipms@kmu.edu.pk)

### Abstract

**Background and Objective:** Vitamin D deficiency and anemia represent synergistic public health challenges among women of reproductive age in South Asia, with profound implications for maternal and child health. Emerging evidence suggests socioeconomic factors, particularly educational attainment, may modify their relationship through complex pathways. This study aimed to investigate the correlation between serum vitamin D (25(OH)D) and hemoglobin (Hb) levels and to examine how educational status influences this relationship among Pakistani women.

**Methods:** This hospital-based cross-sectional study enrolled 134 women aged 18-40 years at Hayatabad Medical Complex, Peshawar. Serum 25(OH)D and hemoglobin were measured using chemiluminescent immunoassay and automated hematology analysis, respectively. Educational status was categorized as illiterate, intermediate, or graduate. Statistical analyses included Pearson correlation, Kruskal-Wallis test, chi-square test, and multiple linear regression using SPSS version 25.

**Results:** The mean age was  $28.5 \pm 7.4$  years. Mean hemoglobin was  $12.0 \pm 1.5$  g/dL, and mean vitamin D was  $27.7 \pm 15.5$  ng/mL. A significant

positive correlation existed between vitamin D and hemoglobin ( $r=0.28$ ,  $p=0.004$ ). Educational status significantly influenced both parameters: graduates exhibited higher median vitamin D (38.7 ng/mL) compared

to illiterate women (24.5 ng/mL,  $p=0.042$ ). Anemia prevalence decreased with increasing education (illiterate: 48.5%, intermediate: 28.1%, graduate: 0%,  $p=0.028$ ). In multiple regression, education level remained an independent predictor of vitamin D status ( $\beta=3.45$ ,  $p=0.008$ ) after controlling sunlight exposure and supplementation.

**Conclusion:** This study confirms a significant association between vitamin D and hemoglobin levels while identifying educational attainment as a critical modifier of this relationship. The findings suggest that nutritional interventions addressing micronutrient deficiencies should be integrated with broader educational and socioeconomic empowerment strategies for women in Pakistan.

## INTRODUCTION

The dual burden of micronutrient deficiencies constitutes a silent epidemic affecting women of reproductive age in low- and middle-income countries. Among these, vitamin D deficiency and anemia represent particularly pervasive challenges with profound implications for maternal and child health outcomes [1]. Vitamin D, traditionally recognized for its skeletal functions, has emerged as a pleiotropic hormone with significant immunomodulatory and erythropoietic properties [2,3]. Concurrently, anemia, predominantly iron-deficiency anemia, affects approximately 40% of non-pregnant women in South Asia, contributing to increased maternal mortality, low birth weight, and impaired cognitive development in offspring [4,5].

Recent epidemiological evidence suggests a compelling biological interplay between vitamin D status and hemoglobin levels. Mechanistically, vitamin D may influence erythropoiesis through direct stimulation of erythroid progenitor cells in bone marrow and indirect modulation of iron homeostasis via hepcidin regulation [6,7]. The vitamin D receptor, abundantly expressed in hematopoietic tissues, mediates these effects through genomic and non-genomic pathways [8]. Despite growing recognition of this association, contextual factors influencing both micronutrient statuses in specific populations remain underexplored.

Socioeconomic determinants, particularly educational attainment, represent critical but often overlooked modifiers of nutritional status. Education influences health literacy, dietary diversity, healthcare-seeking behavior, and implementation of preventive measures [9,10]. In Pakistan, where female literacy rates remain suboptimal, educational disparities may exacerbate existing nutritional inequalities. Previous studies have demonstrated that educated women exhibit better nutritional knowledge, higher dietary diversity, and improved health outcomes [11], but few have examined how education specifically modifies the relationship between vitamin D and hemoglobin.

This study aims to address this knowledge gap by investigating two primary objectives by examining the correlation between serum vitamin D and hemoglobin levels in women of reproductive age in Peshawar and analyzing how educational status modifies this relationship and independently influences both micronutrient parameters. The findings will inform integrated public health strategies that address both biological and socioeconomic determinants of women's nutritional health in Pakistan.

### Materials and Methods

This cross-sectional study was conducted at the Hayatabad Medical Complex (HMC) in Peshawar, Pakistan. HMC serves as a major tertiary care facility catering to a diverse urban and semi-urban population from across Khyber Pakhtunkhwa province. The study received approval from the Institutional Review Board of Hayatabad Medical Complex, and written informed consent was obtained from all participants before enrollment.

The sample size was calculated using the formula  $n = Z^2 \times P(1-P)/d^2$ , with  $Z=1.645$  (90% confidence level),  $P=0.582$ , and  $d=0.07$  (7% margin of error), yielding a minimum required sample of 134 participants. Non-probability convenience sampling was employed to recruit women aged 18-40 years presenting for routine healthcare visits at the outpatient departments. Exclusion criteria included pregnancy or lactation, chronic diseases affecting micronutrient metabolism (such as chronic kidney disease, malignancies, malabsorption syndromes), use of medications known to affect vitamin D or hemoglobin metabolism (including anticonvulsants, corticosteroids, and chemotherapy) and diagnosed hemoglobinopathies.

Data collection involved a multi-method approach. Trained research assistants administered a structured questionnaire in Urdu, the local language, covering demographic characteristics (age, marital status), socioeconomic factors (with educational attainment categorized as illiterate, intermediate [ $\leq 12$  years of schooling], or graduate [ $\geq 14$  years of education]), occupation, and household income), nutritional behaviors (frequency of dairy consumption, use of vitamin D and iron supplements), lifestyle factors (daily sunlight exposure duration categorized as  $<30$ , 30-60, 60-90, or  $>90$  minutes), and relevant medical history. Anthropometric measurements included height and weight measured using standardized equipment, with body mass index (BMI) calculated as weight in kilograms divided by height in meters squared.

For laboratory analysis, 5 mL of venous blood was collected from each participant. Blood for complete blood count was collected in EDTA tubes, while samples for vitamin D analysis were collected in serum gel tubes. All samples were processed within 2 hours of collection. Hemoglobin concentration was measured using a Sysmex XN-1000 automated hematology analyzer. Serum 25-hydroxyvitamin D concentration was quantified using a chemiluminescent immunoassay (DiaSorin Liaison), with quality control maintained using manufacturer-provided controls throughout the analysis. According to established guidelines, vitamin D deficiency was defined as serum 25(OH)D  $<20$  ng/mL, and anemia as hemoglobin  $<12$  g/dL [12,13].

### Statistical Analysis

Data were analyzed using SPSS version 25.0. Continuous variables were presented as means  $\pm$  standard deviations or medians with interquartile ranges, as appropriate, based on the Shapiro-Wilk normality test. Categorical variables were expressed as frequencies and percentages.

The primary relationship between serum 25(OH)D and hemoglobin (Hb) was analyzed using the Pearson correlation coefficient. Differences in micronutrient levels across educational categories (illiterate, intermediate, and graduate) were assessed using the Kruskal-Wallis test, with Dunn's post-

hoc test for pairwise comparisons. Associations between categorical variables (e.g., education and anemia prevalence) were examined using the chi-square test (or Fisher's exact test where appropriate). Multivariate modeling was performed to adjust for confounders. Multiple linear regression was used to identify predictors of serum vitamin D levels, including educational level (coded numerically), sunlight exposure, supplementation status, age, and BMI. Binary logistic regression was employed to determine risk factors for anemia (Hb <12 g/dL), with educational level, vitamin D status, and iron supplementation as key predictors. Model assumptions were verified, and statistical significance was set at  $p < 0.05$ .

## Results

### Baseline Characteristics

The study included 134 women with a mean age of  $28.5 \pm 7.4$  years. Mean BMI was  $23.1 \pm 3.2$  kg/m<sup>2</sup>, indicating a predominantly normal-weight population. Sociodemographic characteristics revealed significant educational disparities: 73.9% were illiterate, 23.9% had intermediate education, and only 2.2% were graduates (Table 1). Correspondingly, 66.4% were unemployed, reflecting limited economic empowerment.

**Table 1: Baseline Characteristics of Study Participants (N=134)**

Characteristic	Category	Frequency (%)
Education	Illiterate	99 (73.9%)
	Intermediate	32 (23.9%)
	Graduate	3 (2.2%)
Occupation	Unemployed	89 (66.4%)
	Student	16 (11.9%)
	Employed	29 (21.6%)
Sunlight Exposure	<30 min	9 (6.7%)
	30-60 min	39 (29.1%)
	60-90 min	60 (44.8%)

Characteristic	Category	Frequency (%)
	>90 min	26 19.4%)

**Nutritional Parameters and Supplementation**

Mean hemoglobin was 12.0 ± 1.5 g/dL, with 44.8% (n=60) meeting criteria for anemia (Hb<12 g/dL). Mean vitamin D level was 27.7 ± 15.5 ng/mL, with 41.0% (n=55) classified as deficient (<20 ng/mL). Supplementation practices were suboptimal: only 15.7% reported vitamin D supplementation and 13.4% reported iron supplementation. Dietary dairy consumption was moderate, with 60.4% consuming dairy products 2-3 times weekly.

**Primary Analysis: Vitamin D-Hemoglobin Correlation**

A significant positive correlation was observed between vitamin D and hemoglobin levels (r=0.28, p=0.004). Participants with vitamin D deficiency had significantly lower mean hemoglobin (11.2 ± 1.3 g/dL) compared to those with sufficient levels (12.6 ± 1.4 g/dL, p<0.001).

Table 2: Association between Vitamin D Status and Anemia

Vitamin D Status	Anemic (Hb<12)	Non-anemic (Hb≥12)	Total	p-value
Deficient (<20 ng/mL)	30 (54.5%)	25 (45.5%)	55	0.012
Sufficient (≥20 ng/mL)	23 (35.9%)	41 (64.1%)	64	

**Secondary Analysis: Influence of Educational Status**

**Education and Vitamin D Levels**

Educational attainment significantly influenced vitamin D status (Kruskal-Wallis H=8.32, df=2, p=0.042). Graduates exhibited the highest median vitamin D levels (38.7 ng/mL [IQR: 32.5-45.2]), followed by intermediate-educated (31.2 ng/mL [20.4-42.1]), and illiterate women (24.5 ng/mL [15.8-35.2]) (Figure 2). Post-hoc Dunn's test revealed significant differences between illiterate and graduate groups (p=0.018).

**Education and Anemia Prevalence**

A significant inverse relationship existed between educational level and anemia prevalence (χ²=7.12, df=2, p=0.028). Among illiterate women, 48.5% were anemic compared to 28.1% of intermediate-educated and 0% of graduates (Table 3).

Table 3: Anemia Prevalence by Educational Level

Education	Total n	Anemic n (%)	Non-anemic n (%)	p-value
Illiterate	99	48 (48.5%)	51 (51.5%)	0.028
Intermediate	32	9 (28.1%)	23 (71.9%)	
Graduate	3	0 (0.0%)	3 (100.0%)	

**Education and Health Behaviors**

Educational status correlated with preventive health behaviors. Graduates reported higher rates of vitamin D supplementation (66.7% vs. 10.1% in illiterate,  $p < 0.001$ ) and more optimal sunlight exposure (>90 minutes daily: 66.7% vs. 15.2% in illiterate,  $p = 0.008$ ).

**Multivariate Analysis**

**Predictors of Vitamin D Levels**

Multiple linear regression revealed that educational level ( $\beta = 3.45$ ,  $p = 0.008$ ), sunlight exposure ( $\beta = 2.89$ ,  $p = 0.003$ ), and vitamin D supplementation ( $\beta = 12.36$ ,  $p < 0.001$ ) were independent predictors of serum vitamin D levels, accounting for 32.4% of the variance ( $R^2 = 0.324$ , Adjusted  $R^2 = 0.298$ ,  $F = 12.28$ ,  $p < 0.001$ ) (Table 4).

Table 4: Multiple Linear Regression for Predictors of Vitamin D Levels

Predictor	$\beta$	SE	T	p-value
Constant	15.24	4.82	3.16	0.002
Education Level	3.45	1.28	2.70	0.008
Sunlight Exposure	2.89	0.94	3.07	0.003
Vitamin D Suppl.	12.36	3.42	3.61	<0.001
Age	-0.08	0.07	-1.14	0.256
BMI	0.21	0.18	1.17	0.245

### Predictors of Anemia

Logistic regression identified vitamin D deficiency (OR=2.21, 95% CI: 1.12-4.38,  $p=0.022$ ), illiteracy (OR=2.85, 95% CI: 1.25-6.50,  $p=0.013$ ), and lack of iron supplementation (OR=2.42, 95% CI: 1.08-5.42,  $p=0.032$ ) as independent risk factors for anemia.

### Discussion

This study provides novel insights into the interplay between micronutrient status and socioeconomic determinants among Pakistani women of reproductive age. Our findings confirm a significant positive correlation between vitamin D and hemoglobin levels while elucidating the pivotal role of educational attainment in modifying this relationship and independently influencing both parameters.

The observed correlation ( $r=0.28$ ,  $p=0.004$ ) between vitamin D and hemoglobin aligns with previous research [14,15] and supports the proposed biological mechanisms. Vitamin D may enhance erythropoiesis through direct stimulation of erythroid progenitor cells via VDR-mediated pathways [6] and indirect modulation of iron homeostasis through hepcidin suppression [7]. Our finding that vitamin D-deficient women had 2.2 times higher odds of anemia reinforces the clinical relevance of this association. These results suggest that vitamin D assessment should be considered in the diagnostic workup of anemia, particularly in regions with high deficiency prevalence.

The novel contribution of this study lies in its demonstration of education as a significant modifier of micronutrient status. The graded relationship between educational attainment and vitamin D levels, with graduates exhibiting 58% higher median values than illiterate women, highlights education as a social determinant of nutritional health. This association persisted after controlling sunlight exposure and supplementation in multivariate analysis ( $\beta=3.45$ ,  $p=0.008$ ), suggesting that education influences vitamin D status through pathways beyond simple behavioral mediators.

Several interconnected mechanisms may explain this educational gradient. First, education enhances health literacy, enabling women to understand the importance of sunlight exposure, dietary diversity, and preventive supplementation [9]. Our data support this, showing that educated women reported better supplementation practices and sunlight exposure. Second, education often correlates with economic empowerment, facilitating access to vitamin D-rich foods and healthcare services [10]. Third, educated women may have greater autonomy in decision-making regarding health behaviors and resource allocation within households [11].

The striking inverse relationship between education and anemia prevalence, with zero cases among graduates compared to nearly half of illiterate women, further emphasizes education's protective role. While vitamin D status contributed to this relationship, education remained an independent predictor in regression models (OR=2.85,  $p=0.013$ ), suggesting additional pathways such as better dietary iron intake, reduced parasitic infections, and improved healthcare utilization.

Our findings align with the conceptual framework of "nutrition transition," wherein socioeconomic development, including education, drives improvements in nutritional status [16]. However, the extreme educational disparities in our sample (74% illiteracy) highlight the urgent need for targeted interventions. These results suggest that micronutrient supplementation programs, while necessary,

may have limited efficacy without simultaneous efforts to improve female education and empowerment.

The study's strengths include its comprehensive assessment of both biological and socioeconomic factors, use of objective laboratory measures, and multivariate analysis controlling for potential confounders. However, several limitations warrant consideration. The cross-sectional design precludes causal inferences, and convenience sampling may limit generalizability. The small number of graduates (n=3) reduces statistical power for this subgroup, though the consistent trends across all analyses strengthen our conclusions. Unmeasured confounders, such as genetic factors, detailed dietary intake, and parasitic infections, may have influenced the results.

Future research should employ longitudinal designs to establish temporal relationships and examine specific pathways through which education influences micronutrient status. Qualitative studies exploring women's knowledge, attitudes, and practices regarding nutrition would provide deeper insights for intervention design. Additionally, intervention trials testing integrated approaches combining micronutrient supplementation with educational components are needed.

### Conclusion

This study demonstrates a significant correlation between vitamin D and hemoglobin levels among Pakistani women of reproductive age and identifies educational attainment as a critical determinant of both micronutrient parameters. The findings underscore the need for integrated public health strategies that address both biological deficiencies and socioeconomic disparities.

### Conflict of interest

The authors declared no conflict of interest.

### References

- Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord.* 2017;18(2):153-165.
- Ismailova A, White JH. Vitamin D, infections and immunity. *Rev Endocr Metab Disord.* 2022;23(2):265-277.
- Demay MB, Pittas AG, Bikle DD, et al. Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2024.
- Stevens GA, Finucane MM, De-Regil LM, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011. *Lancet Glob Health.* 2013;1(1):e16-e25.
- Camaschella C. Iron deficiency. *Blood.* 2019;133(1):30-39.
- Aucella F, Scalzulli RP, Gatta G, et al. Calcitriol increases burst-forming unit-erythroid proliferation in chronic renal failure. *Nephron Clin Pract.* 2003;95(4):c121-c127.
- Bacchetta J, Zaritsky JJ, Sea JL, et al. Suppression of iron-regulatory hepcidin by vitamin D. *J Am Soc Nephrol.* 2014;25(3):564-572.

- Haussler MR, Whitfield GK, Kaneko I, et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int.* 2013;92(2):77-98.
- Nutbeam D. The evolving concept of health literacy. *Soc Sci Med.* 2008;67(12):2072-2078.
- Vorster HH. The link between poverty and malnutrition: A South African perspective. *Health SA Gesondheid.* 2010;15(1):1-6.
- Smith LC, Haddad L. Explaining child malnutrition in developing countries: A cross-country analysis. IFPRI Research Report 111. 2000.
- Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academies Press; 2011.
- World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: WHO; 2011.
- Sim JJ, Lac PT, Liu ILA, et al. Vitamin D deficiency and anemia: a cross-sectional study. *Ann Hematol.* 2010;89(5):447-452.
- Smith EM, Tangpricha V. Vitamin D and Anemia: Insights into an Emerging Association. *Curr Opin Endocrinol Diabetes Obes.* 2015;22(6):432-438.
- Popkin BM. The nutrition transition and its health implications in lower-income countries. *Public Health Nutr.* 1998;1(1):5-21.
- Liu T, Zhong S, Liu L, Liu S, Li X. Vitamin D deficiency and the risk of anemia: a meta-analysis of observational studies. *Ren Fail.* 2015;37(6):929-934.
- Soepnel LM, Mabetha K, Draper CE, et al. A Cross-Sectional Study of the Associations between Biomarkers of Vitamin D, Iron Status, and Hemoglobin in South African Women of Reproductive Age. *Curr Dev Nutr.* 2023;7(5):100072.
- Pereira M, Santos DB. Vitamin D deficiency and anemia in pregnant women: a systematic review and meta-analysis. *Nutr Rev.* 2022;80(3):468-478.
- Janoušek J, Pilařová V, Macáková K, et al. Vitamin D: sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods. *Crit Rev Clin Lab Sci.* 2022;59(8):517-554.