



## Original Article



## Maternal Vitamin A Deficiency in Pregnancy and its Relationship with Maternal and Neonatal Haemoglobin Concentration

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## ABSTRACT

Vitamin A Deficiency (VAD) remains a major nutritional concern, particularly in low-income countries, and is associated with maternal anemia and adverse neonatal outcomes. **Objective:** To assess the correlation between maternal Vitamin A levels and hemoglobin concentrations in both mothers and neonates along with its impact on neonatal health outcomes. **Methods:** A cross-sectional study was conducted at Khyber medical university (Hayatabad Medical Complex, Peshawar), over one year. Pregnant women in their third trimester were recruited based on predefined eligibility criteria. Serum Vitamin A levels were measured using high-performance liquid chromatography (HPLC), and hemoglobin levels were assessed in both maternal and neonatal blood samples. Statistical analyses included t-tests, chi-square tests, and binary logistic regression. **Results:** Among 121 participants, 84% were Vitamin A deficient (<0.70 µmol/L). Maternal hemoglobin was significantly lower in the deficient group (10.38 g/dL vs. 11.83 g/dL,  $p = 0.001$ ), as was neonatal hemoglobin (13.40 g/dL vs. 14.31 g/dL,  $p = 0.001$ ). Low birth weight was more common in the deficient group (31.0% vs. 10.8%), though not statistically significant after Bonferroni correction. Logistic regression confirmed Vitamin A deficiency as an independent predictor of low neonatal hemoglobin (AOR = 3.174,  $p = 0.043$ ). **Conclusions:** Vitamin A deficiency is significantly associated with lower maternal and neonatal hemoglobin levels. These findings highlight the importance of maternal nutrition interventions to prevent anemia-related complications.

## INTRODUCTION

Vitamin A deficiency (VAD) is a significant nutritional concern worldwide, particularly in low- and middle-income countries, affecting approximately 19 million pregnant women globally [1]. VAD during pregnancy not only impacts maternal health but is also associated with an increased risk of adverse neonatal outcomes, including low birth weight, preterm birth, and neonatal anemia [2]. Vitamin A plays a crucial role in immune function, cell growth, and hematopoiesis, the process of blood formation. In regions where dietary intake of Vitamin A is inadequate, its deficiency presents a serious public health concern,

particularly for pregnant women and newborns [3]. The prevalence of VAD among pregnant women varies across different regions, with higher rates reported in Asia and Africa [1]. In Pakistan, studies indicate a notably high prevalence of Vitamin A deficiency among women of reproductive age, raising concerns about its impact on maternal and neonatal health [4, 5]. Poor dietary diversity, low socioeconomic status, and limited healthcare access contribute to this nutritional deficiency, particularly in vulnerable populations. Given that maternal anemia is common in these regions, it is essential to explore the role



of Vitamin A in hemoglobin synthesis and anemia prevention. During pregnancy, the maternal demand for essential nutrients, including Vitamin A, increases to support fetal development and physiological adaptations for childbirth [6]. Low serum retinol levels, indicative of Vitamin A deficiency, may impair red blood cell formation and contribute to maternal anemia [7]. Pregnancy-related anemia is associated with increased risks of low birth weight, preterm delivery, and neonatal health complications. Ensuring adequate Vitamin A intake is therefore critical for maintaining optimal hemoglobin concentrations and reducing anemia in both mothers and neonates [8, 9]. Notably, very limited research has specifically examined the role of maternal Vitamin A deficiency in these neonatal outcomes, highlighting a significant gap in the literature. This study hypothesized that Vitamin A deficiency was associated with lower hemoglobin levels in mothers and neonates and increases the risk of adverse neonatal outcomes, including low birth weight and NICU admission. The primary objective of this research was to assess the correlation between maternal Vitamin A levels and hemoglobin concentrations in both mothers and newborns along with its impact on neonatal health outcomes. Additionally, the study aims to determine the prevalence of Vitamin A deficiency among pregnant women, compare hemoglobin levels between Vitamin A deficient and sufficient groups, and evaluate neonatal outcomes such as low birth weight and NICU admission. However, there is a notable lack of research investigating the direct link between maternal Vitamin A status and hemoglobin concentration in both mothers and neonates, further emphasizing the need for studies in Pakistan.

Vitamin A deficiency during pregnancy remains a major public health issue in low- and middle-income countries, including Pakistan, where poor maternal nutrition contributes to anemia and adverse neonatal health outcomes. Despite the recognized role of Vitamin A in hematopoiesis and fetal development, limited local research has specifically examined the direct relationship between maternal Vitamin A status, maternal and neonatal hemoglobin concentrations, and neonatal outcomes such as low birth weight and NICU admission. This lack of region-specific evidence creates a critical gap in understanding the broader clinical implications of maternal micronutrient deficiencies. Therefore, this study aimed to determine the prevalence of maternal Vitamin A deficiency, evaluate its association with maternal and neonatal hemoglobin levels, and assess its impact on neonatal health outcomes.

## METHODS

This cross-sectional study was conducted at Hayatabad Medical Complex, Peshawar, a tertiary care hospital, affiliated with Khyber Medical University. The study

spanned one year (January 22, 2018 – January 22, 2019) to investigate the association between maternal Vitamin A levels and hemoglobin concentration in both mothers and neonates, along with its impact on neonatal health outcomes. Hayatabad Medical Complex provides maternal and child healthcare services, catering to a diverse population. However, since the study was conducted in a tertiary care setting, there is a possibility of selection bias, as hospital-based populations may differ from the general pregnant population in primary healthcare centers or rural areas. Approval for the study was obtained from the Ethics Review Committee (ERC) of Khyber Medical University (Ref No: DIR/KMU-ASandRB/MV/000737). Informed consent was obtained from all participants, ensuring confidentiality and voluntary participation. Participants were informed about the study's objectives, procedures, and potential risks before enrolment. A convenience sampling method was used, recruiting pregnant women from hospital antenatal and delivery units based on eligibility criteria. The inclusion criteria comprised pregnant women in the third trimester (>28 weeks), willingness to provide blood samples, and the absence of chronic illnesses such as diabetes and hypertension. Exclusion criteria included women with multiple pregnancies, those taking vitamin A supplements, and individuals with pre-existing chronic conditions. The sample size was determined using a standard deviation (SD) of 1.75 g/dL for maternal hemoglobin, based on previous studies (Neves *et al.*, 2019) [9]. Using a 95% confidence level ( $Z = 1.96$ ) and 80% power ( $Z = 0.84$ ), the estimated required sample size per group was 48 participants, totaling 96 participants. Formula Used: where:  $\sigma = 1.75$  g/dL (standard deviation),  $d = 1.0$  g/dL (effect size),  $Z(\alpha/2) = 1.96$  (for 95% CI, two-tailed),  $Z(\beta) = 0.84$  (for 80% power). To account for dropouts and variability, the final sample size was increased to 121 participants. Structured interviews and medical records were used to collect demographic and clinical data, including: Maternal age, education, occupation, monthly income. BMI was calculated using the standard formula (weight in kg/height in  $m^2$ ), and nutritional status was categorized according to WHO classification: BMI  $<18.5$   $kg/m^2$  as malnourished and  $18.5$ – $24.9$   $kg/m^2$  as normal.

**Laboratory Analysis: Vitamin A Measurement (HPLC Protocol).** Blood samples were collected after an overnight fast. Serum Vitamin A levels were measured using High-Performance Liquid Chromatography (HPLC), a precise and reliable method for retinol detection.

**HPLC Protocol:** Serum was extracted using hexane, evaporated under nitrogen gas, and reconstituted in methanol.

**Chromatographic Conditions:** Column: C18 reverse-phase column

**Mobile Phase:** Methanol:water (98:2) **Detection:** UV detector at 325 nm **Retention Time:** ~4.5 minutes.

Participants were classified into two groups based on serum retinol levels: Deficient:  $<0.70 \mu\text{mol/L}$ . Sufficient:  $\geq 0.70 \mu\text{mol/L}$ . Neonatal Hemoglobin Measurement and Consideration of Delivery Factors. Maternal blood samples were collected at the time of admission for delivery, while neonatal cord blood samples were collected immediately after birth using an automated hematology analyzer Sysmex XN-1000 (Sysmex Corporation, Japan). Hemoglobin was measured in grams per deciliter (g/dL). Potential Confounding Factors (Cord Clamping and Delivery Mode). Delayed cord clamping and mode of delivery were not specifically accounted for in neonatal hemoglobin measurements. This represents a study limitation as these factors could influence cord blood hemoglobin levels. Data analysis was performed using SPSS version 24.0. The statistical analysis included calculating means and standard deviations for continuous variables like age, BMI, and gestational age, and frequencies for categorical variables such as parity, education, occupation, monthly income, and nutritional status. T-tests, compare maternal and neonatal hemoglobin levels between Vitamin A Deficient vs. Sufficient groups. Chi-square tests: Analyze associations between Vitamin A status and neonatal outcomes (preterm birth, low birth weight, NICU admission, jaundice). Binary logistic regression: Adjusted for maternal anemia, BMI, socioeconomic status, and parity to control for confounders influencing hemoglobin levels. A p-value  $<0.05$  was considered statistically significant, with Bonferroni correction applied for multiple comparisons.

## RESULTS

The study population had a mean age of 28.00 years (SD = 4.70) and an average BMI of  $24.62 \text{ kg/m}^2$  (SD = 3.393). The mean gestational age among participants was 35.9 weeks (SD = 2.05). In terms of parity, 44.6% of women had one child, while 28.1% were nulliparous. Regarding education, 65.3% had a high school education, and 34.7% attended college. Employment status was nearly evenly split (47.9% employed, 52.1% unemployed). More than half of the

participants (52.1%) had a monthly income between 15,000–25,000 PKR. Nutritional status data indicated that 34.7% of women were malnourished, while 65.3% had normal nutritional status (Table 1).

**Table 1:** Socio-demographic Characteristics of the participants

| Characteristics             | (Mean $\pm$ SD) / Frequency (%) |
|-----------------------------|---------------------------------|
| Age (Years)                 | 28.00 $\pm$ 4.70                |
| BMI (Kg/m <sup>2</sup> )    | 24.62 $\pm$ 3.393               |
| Gestational Age (Weeks)     | 35.9 $\pm$ 2.05                 |
| <b>Parity</b>               |                                 |
| 0                           | 34 (28.1%)                      |
| 1                           | 54 (44.6%)                      |
| 2                           | 17 (14.0%)                      |
| 3                           | 16 (13.2%)                      |
| <b>Education Level</b>      |                                 |
| High School                 | 79 (65.3%)                      |
| College                     | 42 (34.7%)                      |
| <b>Occupation</b>           |                                 |
| Employed                    | 58 (47.9%)                      |
| Unemployed                  | 63 (52.1%)                      |
| <b>Monthly Income (PKR)</b> |                                 |
| <15000                      | 33 (27.3%)                      |
| >15000-25000                | 63 (52.1%)                      |
| >25000-75000                | 25 (20.7%)                      |
| <b>Nutritional Status</b>   |                                 |
| Malnourished                | 42 (34.7%)                      |
| Normal                      | 79 (65.3%)                      |

The majority of participants (84%) were in the Vitamin A deficient group, with a mean vitamin A level of  $0.66 \mu\text{mol/L}$ . Maternal hemoglobin levels were significantly lower in the deficient group ( $10.38 \text{ g/dL}$ ) compared to the sufficient group ( $11.83 \text{ g/dL}$ ,  $p = 0.001$ ). Similarly, neonatal hemoglobin levels were lower in the deficient group ( $13.40 \text{ g/dL}$ ) than in the sufficient group ( $14.31 \text{ g/dL}$ ,  $p = 0.001$ ). These findings suggest an association between Vitamin A deficiency and lower hemoglobin levels in both mothers and their neonates (Table 2).

**Table 2:** Maternal Vitamin A and Hemoglobin Levels by Status with P-Values

| Vitamin A Status                           | Participants (%) | Mean Vitamin A Level ( $\mu\text{mol/L}$ ) | Maternal Hemoglobin (g/dL) | Neonatal Hemoglobin (g/dL) | P-Value (Maternal Hemoglobin and Neonatal Hemoglobin) |
|--|------------------|--|----------------------------|----------------------------|---|
| Deficient ( $<0.70 \mu\text{mol/L}$ )      | 84               | $0.66 \pm 0.04$                            | $10.38 \pm 0.62$           | $13.40 \pm 0.73$           | 0.001   |
| Sufficient ( $\geq 0.70 \mu\text{mol/L}$ ) | 37               | $0.80 \pm 0.05$                            | $11.83 \pm 0.66$           | $14.31 \pm 0.69$           | 0.001   |

Preterm Birth 19.0% of neonates in the Vitamin A deficient group were born preterm, compared to 13.5% in the sufficient group. However, this difference was not statistically significant ( $p = 0.459$ , Bonferroni-corrected  $p = 1.836$ ). The odds ratio (OR = 0.664, 95% CI: 0.224–1.972) further indicates no significant association (Table 3)

Low Birth Weight ( $<2.5 \text{ kg}$ ) 31.0% of neonates in the Vitamin A deficient group had low birth weight, compared to 10.8% in the sufficient group. The association was statistically significant before Bonferroni correction ( $p = 0.018$ ). However, after correction, it was no longer significant (corrected  $p = 0.072$ ). The odds ratio (OR = 2.863, 95% CI: 1.076–7.621) suggests a potential but borderline association (Table 3). NICU Admission was slightly higher in neonates from Vitamin A deficient mother (19.0%) compared to the sufficient group (10.8%). This difference was not statistically significant ( $p = 0.261$ , Bonferroni-corrected  $p = 1.044$ ). The odds ratio (OR = 0.515, 95% CI: 0.160–1.663) confirms the lack of a significant relationship

(Table 3). Jaundice 9.5% of neonates in the deficient group had jaundice, compared to 10.8% in the sufficient group. The difference was not statistically significant ( $p = 0.827$ , Bonferroni-corrected  $p = 3.308$ ). The odds ratio (OR = 1.152, 95% CI: 0.324–4.092) also supported the lack of a significant association (Table 3).

**Table 3:** Neonatal Outcomes by Maternal Vitamin A Status with Bonferroni-Corrected p-Values

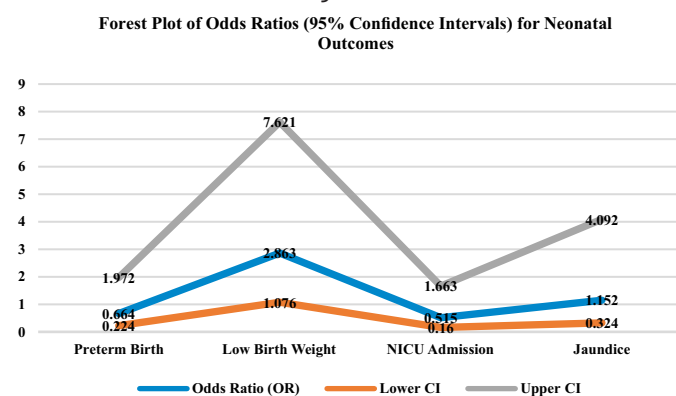
| Neonatal Outcome          | Deficient Frequency (%) | Sufficient Frequency (%) | p-value (Before Correction) | p-value (After Bonferroni Correction) | 95% CI for Odds Ratio (AOR) | Significant After Correction? |
|---------------------------|-------------------------|--------------------------|-----------------------------|---------------------------------------|-----------------------------|-------------------------------|
| Preterm Birth             | 16 (19.0%)              | 5 (13.5%)                | 0.459                       | 1.836                                 | (0.224 - 1.972)             | No                            |
| Low Birth Weight (<2.5kg) | 26 (31.0%)              | 4 (10.8%)                | 0.018                       | 0.072                                 | (1.076 - 7.621)             | No (borderline)               |
| NICU Admission            | 16 (19.0%)              | 4 (10.8%)                | 0.261                       | 1.044                                 | (0.160 - 1.663)             | No                            |
| Jaundice                  | 8 (9.5%)                | 4 (10.8%)                | 0.827                       | 3.308                                 | (0.324 - 4.092)             | No                            |

To determine whether Vitamin A deficiency independently affects neonatal hemoglobin levels, binary logistic regression was performed, adjusting for maternal anemia, socioeconomic status, dietary intake, and BMI. Vitamin A deficiency was significantly associated with low neonatal hemoglobin levels (AOR = 3.174, 95% CI: 1.038–9.699,  $p = 0.043$ ). However, maternal hemoglobin, BMI, gestational age, parity, and socioeconomic factors were not significantly associated with neonatal hemoglobin levels (Table 4).

**Table 4:** Logistic Regression Analysis for Low Neonatal Hemoglobin

| Variables                                    | Adjusted Odds Ratio (AOR) | 95% Confidence Interval (CI) | p-value | Significant |
|--|---------------------------|------------------------------|---------|-------------|
| Vitamin A Deficiency                         | 3.174                     | (1.038 - 9.699)              | 0.043   | Yes         |
| Maternal Hemoglobin (g/dL)                   | 1.088                     | (0.730 - 1.624)              | 0.678   | No          |
| BMI (Kg/m <sup>2</sup> )                     | 1.047                     | (0.929 - 1.180)              | 0.454   | No          |
| Gestational Age (Weeks)                      | 0.895                     | (0.727 - 1.102)              | 0.297   | No          |
| Parity                                       | 0.760                     | (0.500 - 1.155)              | 0.199   | No          |
| Education Level (College vs. High School)    | 0.899                     | (0.383 - 2.110)              | 0.807   | No          |
| Occupation (Employed vs. Unemployed)         | 1.217                     | (0.549 - 2.697)              | 0.629   | No          |
| Monthly Income (PKR 15000-25000 vs. <15000)  | 1.095                     | (0.332 - 3.618)              | 0.881   | No          |
| Monthly Income (PKR 25000-75000 vs. <15000)  | 0.643                     | (0.215 - 1.924)              | 0.430   | No          |
| Nutritional Status (Malnourished vs. Normal) | 1.765                     | (0.741 - 4.204)              | 0.200   | No          |

The forest plot shows the odds ratios (OR) and 95% confidence intervals (CI) for neonatal outcomes associated with maternal Vitamin A deficiency. Low birth weight has the highest OR (2.863) with a wide CI (1.076–7.621), suggesting a borderline association. Preterm birth (OR = 0.664, CI: 0.224–1.972), NICU admission (OR = 0.515, CI: 0.160–1.663), and jaundice (OR = 1.152, CI: 0.324–4.092) show no significant association as their confidence intervals include 1.



**Figure 1:** Forest plot of odds ratios (OR) and 95% confidence intervals (CI) for neonatal outcomes associated with maternal Vitamin A deficiency

## DISCUSSION

This study investigated the relationship between maternal VAD and hemoglobin concentration in both mothers and neonates, along with its impact on neonatal health outcomes. This study found a high prevalence of Vitamin A

deficiency (84%), which was consistent with previous studies conducted in low- and middle-income countries, particularly South Asia, where Vitamin A deficiency remains a major public health concern [10, 11]. Reports from similar settings have documented deficiency rates ranging from 50% to 85% in pregnant women, largely due to limited dietary diversity, micronutrient deficiencies, and socioeconomic constraints affecting nutrition during pregnancy [12, 13]. While these findings align with regional estimates, it is important to consider selection bias, as this study was conducted in a tertiary hospital setting, where pregnant women seeking care may be at higher risk of nutritional deficiencies due to existing health concerns. As a result, the true prevalence of Vitamin A deficiency in the general population of pregnant women may be lower than what was observed in the sample. Mothers with Vitamin A deficiency had significantly lower mean hemoglobin levels than those with sufficient Vitamin A ( $p = 0.001$ ). This finding supports the biological role of Vitamin A in hematopoiesis, as it contributes to red blood cell production and iron

mobilization. Vitamin A deficiency has been linked to impaired hemoglobin synthesis and an increased risk of anemia, which can lead to maternal complications such as fatigue, increased infection susceptibility, and adverse pregnancy outcomes [14, 15]. Similarly, neonates born to Vitamin A deficient mothers had significantly lower hemoglobin levels ( $p = 0.001$ ), suggesting a possible intergenerational effect of maternal nutritional status on neonatal anemia. Neonatal anemia has been associated with higher risks of infection susceptibility and developmental delays [16]. Given that neonatal hemoglobin levels are critical for early growth and immunity, addressing maternal Vitamin A deficiency may contribute to improved neonatal outcomes. Among neonatal health indicators, low birth weight was significantly associated with maternal Vitamin A deficiency before Bonferroni correction ( $p = 0.018$ ), with a higher prevalence of low birth weight in neonates of deficient mothers (31.0%) compared to those of sufficient mothers (10.8%). However, after Bonferroni correction ( $p = 0.072$ ), the association became borderline significant, suggesting a potential relationship that warrants further investigation. Since maternal micronutrient deficiencies have been linked to impaired fetal growth, Vitamin A deficiency could contribute to fetal underdevelopment [17], although additional longitudinal studies are needed to confirm a direct causal link. For preterm birth ( $p = 0.459$ ), NICU admission ( $p = 0.261$ ), and jaundice ( $p = 0.827$ ), no statistically significant associations with maternal Vitamin A status were found. However, preterm birth rates were slightly higher among Vitamin A deficient mothers (19.0% vs. 13.5%), and NICU admissions followed a similar pattern (19.0% vs. 10.8%). While these findings were not statistically significant, they align with existing research that maternal micronutrient deficiencies may contribute to pregnancy complications [18-20]. The strengths of this study include its focus on a vulnerable population, the use of laboratory-confirmed Vitamin A levels, and a comprehensive analysis of maternal and neonatal health outcomes.

However, certain limitations must be acknowledged. Since this study was conducted in a tertiary hospital, specifically at Hayatabad Medical Complex, Peshawar, there is a possibility of selection bias. Pregnant women seeking care in hospital settings often have existing health concerns, which may have contributed to the higher observed rates of Vitamin A deficiency compared to the general population. Future studies incorporating broader nutritional assessments will be essential to better understand these relationships.

## CONCLUSIONS

This study demonstrated a significant association between maternal Vitamin A deficiency and lower hemoglobin levels in both mothers and neonates, as well as

a potential link to low birth weight. While other neonatal outcomes (preterm birth, NICU admission, jaundice) were not significantly associated with Vitamin A status, the findings emphasize the importance of addressing Vitamin A deficiency during pregnancy. Incorporating maternal nutrition interventions, including Vitamin A supplementation and dietary improvements, into antenatal care programs could help reduce deficiency-related complications and improve birth outcomes. Further research is required to confirm these associations and identify the most effective strategies for preventing Vitamin A deficiency in pregnant populations.

## Authors' Contribution

Conceptualization: SAK

Methodology: SJS

Formal analysis: R

Writing and Drafting: SJS, RM, SAK, BSH, NJ

Review and Editing: SJS, RM, SAK, BSH, NJ

All authors approved the final manuscript and take responsibility for the integrity of the work

## Conflicts of Interest

All the authors declare no conflict of interest.

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