

ASSOCIATION OF VITAMIN D WITH SERUM CALCIUM AND HEMOGLOBIN IN THIRD- TRIMESTER PREGNANT FEMALES

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Abstract

Introduction: vitamin D is the most important vitamin and play vital role in reproductive health. This cross-sectional study is done to evaluate the prevalence of Vitamin D deficiency among pregnant women in third trimester and its relation with age, serum calcium and hemoglobin. The study was carried out at the Department of Physiology, University of Sindh, Jamshoro, from August 2023 to May 2024.

Methodology: 100 pregnant women (20–37 years) in the third trimester recruited from different hospitals of Hyderabad, Sindh, were participated. Vitamin D was determined using a Spectrum ELISA kit, and serum calcium and hemoglobin were measured with the Roche Cobas 6000. Data analysis: Analysis was conducted using SPSS 21.

Results: Mean age of the participants was 26.96 ± 4.37 years. The prevalence of Vitamin D deficiency (<20 ng/ml) was 62%, with 34% insufficient and only 4% sufficient; there was a significant negative correlation between maternal age and Vitamin D $r = -0.331$, $p = 0.019$. Besides, Vitamin D also established strong positive correlations with serum calcium ($r = 0.724$, $p < 0.001$) and hemoglobin ($r = 0.663$, $p < 0.001$), wherein the deficient group has the lowest mean for hemoglobin (8.45 ± 0.68 g/dl).

Conclusion: On the basis of our research we conclude that Vitamin D deficiency is highly prevalent among pregnant women in Sindh. Since low Vitamin D has a strong correlation with hypocalcemia and maternal anemia, it can be deduced that Vitamin D is involved in mineral homeostasis as well as erythropoiesis.

INTRODUCTION

Vitamin D is a powerful fat-soluble vitamin and steroid hormone that has a major function in controlling calcium and phosphate balance. It is produced inside the body when ultraviolet B rays (UVB) light (290–315 nm) goes through the skin layer, changing provitamin D to pre-vitamin D₃, which later changes by heat action into vitamin D₃¹. Other than sunlight, people get vitamin D

from outside sources like fatty fish, egg yolks, and fortified foods or supplements but these are usually not enough to fulfill medical needs during pregnancy².

Vitamin D goes through two crucial steps of hydroxylation for activation. The first step takes place in the liver where it is converted by 25-hydroxylase enzyme to 25-hydroxyvitamin D

[25(OH)D]³. This is followed by conversion in kidneys by 1 α -hydroxylase into its biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)₂D]⁴. The active form is essential to stimulate intestinal calcium absorption and bone mineralization among other roles. Moreover, there has been growing recognition of the autocrine and paracrine functions fulfilled by vitamin D in cell proliferation immune response and apoptosis⁵.

During pregnancy, Vitamin D requirements increase significantly to support the process of fetal development. The input demand for active vitamin D increases by 50-100% because of an unmatching mechanism of the Parathyroid Hormone (PTH) among the related physiological changes⁶. Though the maternal body adjusts by providing intestinal calcium absorption, actual provision during the third trimester when the accretion in fetal bones is at its peak, deficiency results in several side effects. If Vitamin D and calcium content in a mother are low, then that in a neonate will be low as well placing it at more risk from hypocalcemia and retarded bone growth⁷.

Vitamin D is of great significance; its inadequacy is a major health concern around the world, particularly in Pakistan. Dressing habits and less outdoor activity led to an overwhelming percentage of the population with hypovitaminosis D. New regional studies indicated that this deficiency could also be related to hematological markers, particularly hemoglobin, thus explaining the high prevalence of maternal anemia in the Sindh region⁸.

METHODOLOGY

This cross sectional study took place at Department of Physiology, University of Sindh, Jamshoro. The total number of 100 pregnant females in their third trimester recruited from different hospitals in Hyderabad, Sindh.

Women aged 20 to 40 years who were in the third trimester of pregnancy were enrolled in the study. To ensure reliability of metabolic measurements, exclusion criteria were carefully implemented. Women with a known history of diabetes mellitus, thyroid disorders,

cardiovascular disease, or renal disease prior to the present pregnancy were not include⁹.

Ethical guidelines were in accordance with the declaration of Helsinki¹⁰. Each participant underwent clinical assessment, and written informed consent was taken following a brief explanation of the study objectives.

Sample Collection

From each participant, 5 ml of venous blood was drawn under aseptic conditions. Samples were separated into an EDTA tube for hematology tests and a gel tube for biochemical assays.

Hematological Analysis: Hemoglobin (Hb) was estimated with an automated Coulter counter according to standard laboratory methods.

Biochemical Analysis: Serum calcium was run on the Roche Cobas 6000 chemistry analyzer.

Vitamin D Estimation: Concentrations of Serum 25-hydroxyvitamin D [25(OH)D] were analyzed by competitive Enzyme-Linked Immunosorbent Assay (ELISA) based on the method described by the manufacturer (Spectrum Vitamin D Kit). Hence, that cataloging was done as per the Endocrine Society clinical practice guideline¹².

Data analysis was done with SPSS version 21. Descriptive statistics, mean, and standard deviation (\pm SD) were worked out for all quantitative variables. The relationship between vitamin D, serum calcium, and hemoglobin levels was determined using Pearson's correlation coefficient (r). A p-value less than 0.05 were taken to denote statistical significance.

RESULTS

100 pregnant women in the third trimester have been chosen. The age of the subjects ranged between 20 to 37 years. To see trends relating to age, participants were grouped into three different categories. (**Table I**). The highest frequency of the subject came from the 26–30 years age group (n = 42, 42%), this being essentially the main reproductive period followed by a 20–25 years age group (n = 34, 34%). The least number of subjects above 30 years of age were more than

that of the rest - only about twenty-four percent belong to this category. The mean age overall for

this population under study emerged as that would be found out at 26.96 ± 4.37 years.

Table I: Demographic Characteristics and Mean Age of Study Subjects (N = 100)

| Age Group (Years) | Number of Subjects (n) | Percentage (%) | Mean Age (Years \pm SD) |
|-------------------|------------------------|----------------|------------------------------------|
| 20 - 25 | 34 | 34% | 22.18 ± 1.63 |
| 26 - 30 | 42 | 42% | 27.43 ± 1.33 |
| > 30 | 24 | 24% | 32.92 ± 1.84 |
| Total | 100 | 100% | 26.96 ± 4.37 |

Physiological requirements for Vitamin D increase substantially in the third trimester to support fetal development. Mean levels of Vitamin D by all age groups in this study were below clinically recommended standards. A significant negative correlation ($r = -0.331$, $p = 0.019$) was found between maternal age and serum Vitamin D levels, so that the lowest mean

levels were found in subjects older than 30 years of age; 17.72 ± 9.93 ng/ml.

After grouping, most of the people were in the deficient category ($n = 62$, 62%) with mean levels as low as 15.43 ± 3.32 ng/ml. Only a minor part (4%) got to be in the sufficient status. The detailed spread and clinical class are given in Table II.

Table II: Serum Vitamin D Concentration and Clinical Status Classification (N = 100)

| Parameter | Category | n (%) | Vitamin D (ng/ml \pm SD) | Min - Max |
|-------------------------|---------------|------------|----------------------------|---------------|
| Maternal Age Group | 20 - 25 Years | 34 (34%) | 25.48 ± 8.29 | 10.67 - 42.27 |
| | 26 - 30 Years | 42 (42%) | 19.28 ± 6.02 | 9.40 - 32.58 |
| | > 30 Years | 24 (24%) | 17.72 ± 9.93 | 8.11 - 43.11 |
| Vitamin D Status | Deficient | 62 (62%) | 15.43 ± 3.32 | 8.11 - 19.67 |
| | Insufficient | 34 (34%) | 28.64 ± 3.67 | 21.09 - 34.22 |
| | Sufficient | 4 (4%) | 42.69 ± 0.60 | 42.27 - 43.11 |
| Total Population | - | 100 (100%) | 21.01 ± 8.38 | 8.11 - 43.11 |

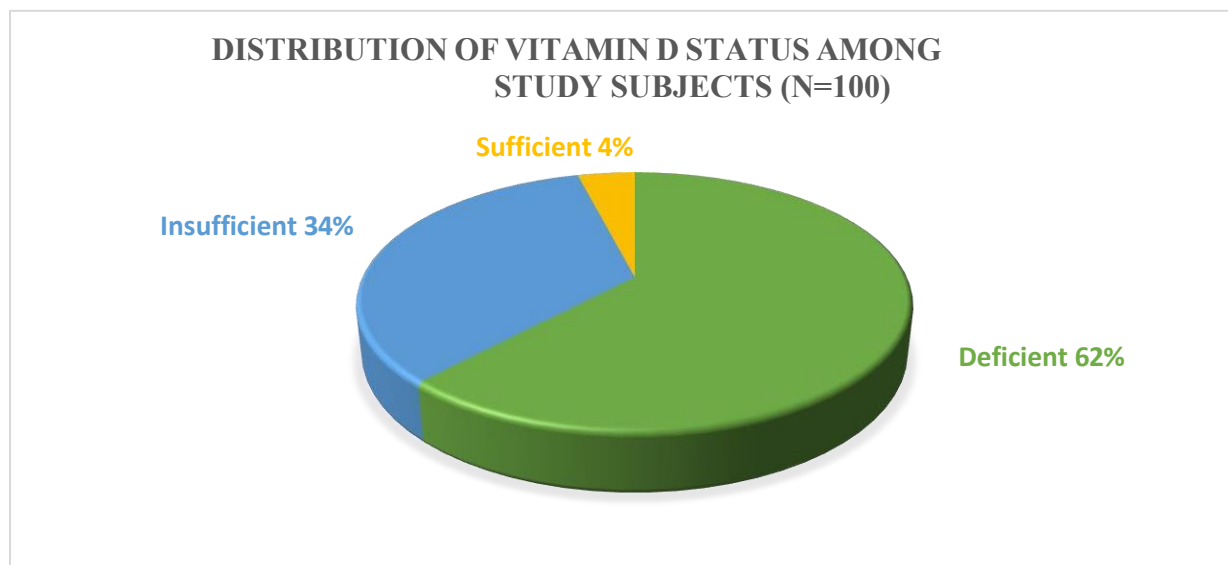


Figure 2: Distribution of Vitamin D Status among Study Subjects (N=100)

Calcium absorption by the mother reaches a peak during the third trimester so that adequate amounts of calcium can be supplied to meet fetal bone development. Mean values for serum calcium in this study for all groups were within clinically normal limits (8.2 - 9.7 mg/dl) but predominantly falling into the lowest quartile. There is no significant relationship between age

and levels of calcium among women, $p > 0.05$. However, another highly significant positive relationship was found between Vitamin D and calcium at $r = 0.724$, $p < 0.001$. This group which is deficient in Vitamin D shows mean levels of calcium at the lowest value (8.27 ± 0.34 mg/dl) further supporting biologically that absorption for calcium depends on the status of Vitamin D.

Table III: Serum Calcium Levels (mg/dl) Distributed by Age and Vitamin D Status (N = 100)

| Variable | Category | n | Serum Calcium (mg/dl ± SD) |
|--------------------|---------------|-----|----------------------------|
| Maternal Age Group | 20 - 25 Years | 34 | 8.61 ± 0.62 |
| | 26 - 30 Years | 42 | 8.39 ± 0.35 |
| | > 30 Years | 24 | 8.51 ± 0.48 |
| Vitamin D Status | Deficient | 62 | 8.27 ± 0.34 |
| | Insufficient | 34 | 8.83 ± 0.50 |
| | Sufficient | 4 | 9.07 ± 0.14 |
| Total Population | — | 100 | 8.49 ± 0.49 |

The results proven that individuals of all age groups have low to moderate anemia. This is since their hemoglobin levels are below the normal value for the third trimester of pregnancy. A test for statistical correlation between maternal age and hemoglobin level returned a value of $r = -0.154$, $p = 0.286$, hence proving that variation of anemia with age was immaterial and thus rendered a uniform prevalence across all study populations.

A highly significant positive correlation between serum Vitamin D levels and hemoglobin

concentration was noted ($r = 0.663$, $p < 0.001$). As seen in **Table IV**, the mean hemoglobin value of those patients classified as Vitamin D deficient was low (8.45 ± 0.68 g/dl), increasing significantly in those with normal values of this vitamin (10.12 ± 0.43 g/dl). This finding goes a long way to support the fact that inadequate levels of Vitamin D may be a major causative factor for maternal anemia in its severest form during the third trimester.

Table IV: Mean Hemoglobin Levels (g/dl) Distributed by Age and Vitamin D Status (N = 100)

| Variable | Category | N | Hemoglobin (g/dl \pm SD) |
|--------------------|---------------|-----|----------------------------|
| Maternal Age Group | 20 - 25 Years | 34 | 9.15 \pm 0.79 |
| | 26 - 30 Years | 42 | 8.70 \pm 0.85 |
| | > 30 Years | 24 | 8.83 \pm 0.90 |
| Vitamin D Status | Deficient | 62 | 8.45 \pm 0.68 |
| | Insufficient | 34 | 9.52 \pm 0.57 |
| | Sufficient | 4 | 10.12 \pm 0.43 |
| Total Population | — | 100 | 8.88 \pm 0.84 |

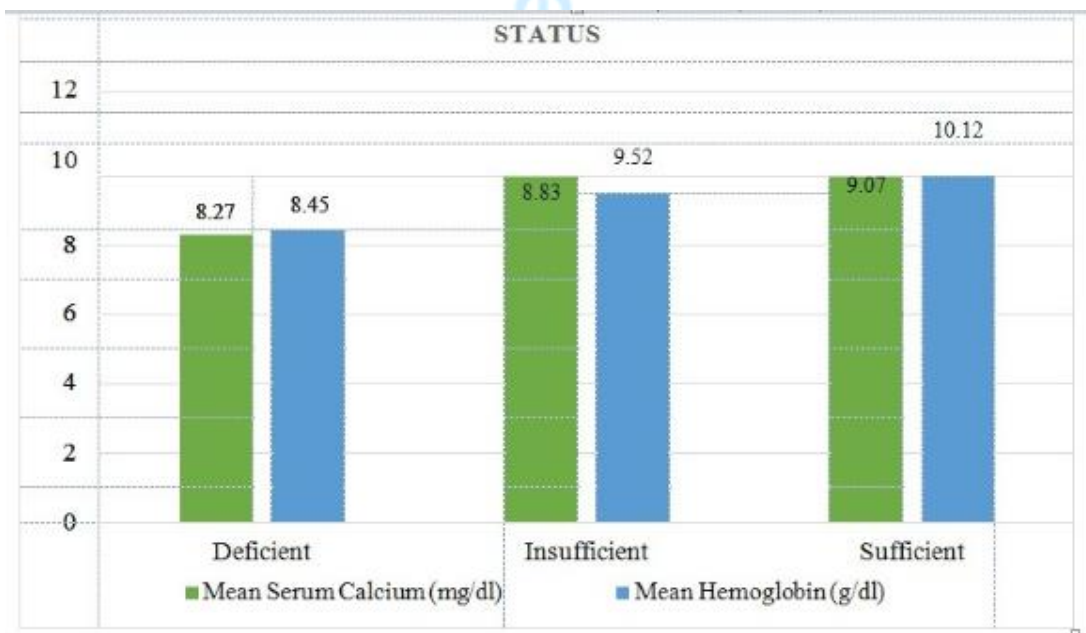


Figure 3: Mean Serum Calcium (mg/dl) and Hemoglobin (g/dl) Levels categorized by Vitamin D Status

Table V presents the results of Pearson correlation analysis between maternal age, Vitamin D, calcium, and hemoglobin. It was only the relationship between maternal age and Vitamin D that turned out to be statistically significant and negative ($r = -0.331$, $p = 0.019$). This finding leads to the inference that older pregnant women will generally have low levels of Vitamin D. The study found that there was no significant relationship

between age and serum calcium ($r = -0.098$, $p = 0.498$) or hemoglobin ($r = -0.154$, $p = 0.286$). Most importantly, Vitamin D showed a highly significant positive correlation with both serum calcium and hemoglobin levels ($r = 0.724$, $p < 0.001$; $r = 0.663$, $p < 0.001$) respectively. This denotes that Vitamin D can be an effective indicator of the status of calcium and hematological health in the body during the third trimester.

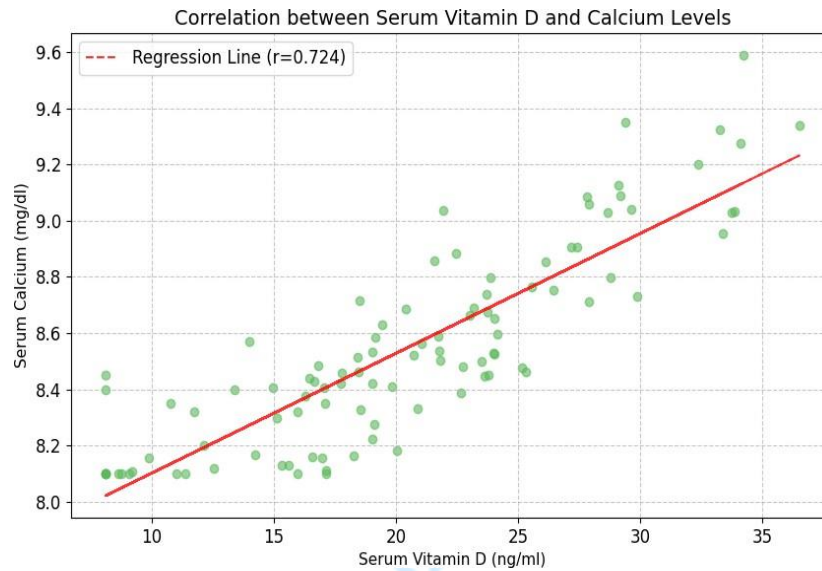


Figure 4: Pearson Correlation between Serum Vitamin D (ng/ml) and Serum Calcium (mg/dl)

Table V: Correlation Matrix of Maternal Age and Biochemical Parameters (N=100)

| Parameters | Age | Vitamin D | Calcium | Hemoglobin |
|-----------------|---------|-----------|---------|------------|
| Age | 1 | -0.331* | -0.098 | -0.154 |
| Sig. (2-tailed) | — | 0.019 | 0.498 | 0.286 |
| Vitamin D | -0.331* | 1 | 0.724** | 0.663** |
| Sig. (2-tailed) | 0.019 | — | 0.000 | 0.000 |
| Calcium | -0.098 | 0.724** | 1 | 0.544** |
| Sig. (2-tailed) | 0.498 | 0.000 | — | 0.000 |
| Hemoglobin | -0.154 | 0.663** | 0.544** | 1 |
| Sig. (2-tailed) | 0.286 | 0.000 | 0.000 | — |
| N | 100 | 100 | 100 | 100 |

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Vitamin D deficiency continues to be a major public health concern in Pakistan, especially among pregnant women. Requirements increase nearly twice during the third trimester for the purpose of fetal skeletal mineralization as well as to maintain good health of the mother¹².

In the current study, the 26–30 years age group has been noted as the most significantly represented demographic with a share of 42%. This reveals the dominant reproductive period in our population. There is a major finding with an assessment of the relationship between maternal age and Vitamin D levels which returned a significant negative correlation ($r = -0.331$, $p = 0.019$). More specifically, deficiency severity was highest among those above 30 years old (Mean: 17.72 ± 9.93 ng/ml). This trend indicates that advancing maternal age may be associated with an increased risk of hypovitaminosis D, potentially owing to cumulative nutritional depletion or limited sun exposure^{13, 14, 15}.

A 62% deficiency rate was recorded, with a further 34% being inadequate. Only 4% of the participants reported adequate levels of Vitamin D. Recent statistics from Zimbabwe can be compared with these results¹⁶ and other non-African cohorts where the deficiency rate varies between 10% and 90%. Such a high incidence in our study area (Jamshoro/Sindh) can be attributed to cultural garmenting, unavailability of outdoor activity, and fortified dietary sources which is the same case in other regional studies as well¹⁷.

Serum calcium in all age groups, though within the clinical normal range (8.2-9.7 mg/dl), had mean values clustered in the lowest quartile. A highly significant positive correlation ($r = 0.724$, $p < 0.001$) between Vitamin D and calcium proves the biological dependence of calcium absorption on the status of Vitamin D¹¹. In the third trimester, if a surge fails to be induced by Vitamin D results in borderline hypocalcemia which may have effects on fetal bone density as well as maternal bone health⁷.

A statistically significant positive correlation was observed between vitamin D and hemoglobin levels ($r = 0.663$, $p < 0.001$). Subjects classified as

deficient demonstrated significantly lower mean hemoglobin concentrations (8.45 ± 0.68 g/dl) compared to that in the sufficient group. This relationship brings to light a function for Vitamin D in erythropoiesis or iron metabolism¹⁸. From this finding, since both conditions are salient public health issues in Pakistan, it advocates for dual supplementation in the third trimester as an intervention to improve maternal and neonatal outcomes^{9, 15, 17}.

CONCLUSION

This study sheds light on the condition of Vitamin D among pregnant women in their third trimester who are residents of this area, indicating a major public health issue. Results revealed a high level of Vitamin D deficiency (62%) and insufficiency (34%) with only an insignificant percentage (4%) having sufficient levels.

The study proves that Vitamin D levels are greatly influenced by maternal age. Also, Vitamin D is the major determinant of serum calcium and hemoglobin levels. There is no direct relationship

found between maternal age and either calcium or hemoglobin. However, the strong positive correlation of Vitamin D with serum calcium ($r = 0.724$) and with hemoglobin ($r = 0.663$; $p < 0.001$) highlights more explicitly its important function in mineral homeostasis as well as erythropoiesis support during late pregnancy.

To avoid maternal bone resorption, borderline hypocalcemia, and exacerbation of anemia, it is safe to institute the practice of routine screening for Vitamin D during antenatal visits. This will include Vitamin D supplementation as part of a targeted strategy and dietary fortification together with safe sunlight exposure to ensure that serum levels remain within recommended clinical ranges for optimal maternal and fetal outcomes.

LIMITATION OF STUDY

The small sample size, along with the study being conducted at a single center, limits the broader applicability of the results

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZUM and JAZ: Data acquisition, data analysis, critical review, approval of the final version to be published.

ZUM, JAZ, FM, BK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ZUM, JAZ, FM, BK, AM, and SS: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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