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ORIGINAL RESEARCH

Vitamin D_3 levels in anemic and non-anemic rural pregnant women- A case control study

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ABSTRACT

Introduction: Vitamin D_3 deficiency, along with iron and other nutrient deficiencies such as carbohydrates, proteins, vitamins, and minerals, can influence maternal and fetal health outcomes. The aim of this study was to assess Vitamin D_3 levels in anemic and non-anemic rural pregnant women –A case control study. **Methods:**This one-year case-control study at Bhagat Phool Singh Medical College involves 400 rural patients aged 21-35. Simple random sampling is used. Participant's medical histories, BMI, and nutritional status are assessed. Pregnant women's, serum ferritin, and vitamin D levels are measured, classifying them into anemic (<10gm/dl) and non-anemic (>10gm/dl) groups. **Results:** The mean ages for Group 1 and Group 2 were 24.63 ± 4.17 and 25.12 ± 3.97 years, respectively. The study found significant differences in mean Vitamin D levels between Group 1(13.42 ng/mL ± 8.94) and Group 2 (18.78 ng/mL ± 10.66) with a P value of 0.001. Vitamin D levels across different ferritin categories, significant differences were observed (P = 0.03). Participants with vitamin D deficiency (< 20 ng/mL) showed higher percentages of ferritin levels below 50 ng/mL (78.7%) compared to those with insufficient (72.1%) and normal (60.7%) vitamin D levels. Analysis of supplemental interventions showed no significant difference in the use of vitamin D tablets along with calcium supplements (P value = 0.22) or iron tablets/syrup (P value = 0.22). Vitamin D injections were administered to 7.5% of Group 1 and 11.5% of Group 2, with no statistically significant distinction (P value = 0.17). **Conclusion:** The primary objective of the study was to evaluate and compare serum vitamin D3 levels between anemic and non-anemic rural pregnant women, aiming to identify significant differences. Secondary objectives included assessing vitamin D3 deficiency incidence and exploring the association between vitamin D3 and serum ferritin levels, aiming to understand their impact on maternal health and anemia. The study highlights vitamin D deficiency as a potential risk factor for anemia in pregnant women, advocating for interventions to optimize maternal vitamin D levels and improve hemoglobin status **Keywords:** Rural pregnant women, Vitamin D, Anemia.

INTRODUCTION

The literature reports that nutritional anemia and deficient vitamin D levels are prominent worldwide, specifically in developing nations. The reported prevalence of anemia in developing countries is 51% and in India is 65-75% **(1)**. The global prevalence of vitamin D deficiency or insufficiency ranges between 54-100% to 39-76% respectively and in India is 40-99% in pregnant women **(2)**. The requirement of vitamin D during pregnancy and lactation, maternal vitamin D, and calcium metabolism undergo significant adaptations to meet the increased demands for fetal bone development **(3)**. The fetus accumulates substantial calcium, particularly in the third trimester, leading to enhanced maternal calcium absorption and urinary excretion to maintain stable serum calcium levels **(4)**. Plasma levels of 1,25-dihydroxy vitamin D [$1,25(OH)_2D$] double early in pregnancy, peak in the third trimester, and normalize during lactation **(5-6).** Prenatal vitamin D deficiency is associated with increased risks of pre-eclampsia, small for gestational age infants, mood disorders, gestational diabetes, iron deficiency anemia, neonatal hypocalcemia, impaired fetal bone development, and low neonatal vitamin D levels **(7)**. Anemia is a major global health issue affecting young children and pregnant women in both developing and developed countries. Adverse pregnancy outcomes, such as gestational diabetes,

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polyhydramnios, preterm birth, low birth weight, and neonatal complications, are significantly higher in anemic antenatal women **(8)**. Literature reports that vitamin D influences hemoglobin levels by promoting erythropoiesis and counteracting inflammation. vitamin D is synthesized in the skin by UV rays, a 30 to 35-minute exposure to sunlight is considered sufficient for increasing vitamin D3 levels. The best time for vitamin D synthesis is between 11 a.m. and 2 p.m**(9).**Vitamin D3 influences hemoglobin levels and iron metabolism, which are crucial during the pro-inflammatory state of pregnancy The active form, $1,25(OH)_2D$, enhances the effects of erythropoietin and stimulates erythroid precursor proliferation. Inflammation, which is common in pregnancy, raises proinflammatory cytokines and hepcidin levels, suppressing erythropoiesis and reducing iron availability **(10).** Vitamin D helps reduce these cytokines and downregulates hepcidin, thereby increasing iron export via ferroportin and supporting erythropoiesis**(11)**. Therefore, maintaining adequate vitamin D3 levels during pregnancy is critical to prevent deficiencies in both mothers and infants. In India, factors contributing to vitamin D deficiency include dark-pigmented skin, reduced sunlight exposure, pollution, clothing practices, and dietary deficits, especially in unplanned pregnancies and rural settings where sunlight exposure is presumed to be adequate **(12)**. Literature reports a correlation between anemia and vitamin D levels, suggesting that vitamin D enhances erythropoiesis **(13-14).**This study was planned to study levels of vitamin D3 in anemic and non-anemic pregnant women to know the association between anemia and vitamin D levels.

MATERIAL AND METHODS

This case control study was conducted at the Department of Obstetrics and Gynaecology, Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonipat, over one year. The study sample included all pregnant women admitted in labour rooms in labour in the age group of 21-35 years residing in rural areas, who agreed to participate in the study. Pregnant women with HIV infection, hypothyroidism, chronic renal and liver diseases, seizure disorders under medication, mental disabilities, coagulation disorders, and refusal to participate were excluded from the study. The sample size is based upon a case-control study with equal allocation for finding the association, the hypothesis of odd ratio taking exposure in the control group 21% with 1.9 odds ratio at 80% power and 5% alpha error. The required sample size in each group is 196 i.e. 392. For simplicity in the calculation, the total sample size was rounded to 400 and 200 in each group. All IPD pregnant women admitted to the labour room for delivery and given consent fulfilling inclusion criteria will be subjected to detailed history taking, past medical, and personal details, socioeconomic, lifestyle, dietary pattern, routine exposure to sunlight, and obstetrics history, will be taken.

All pregnant women participating in the study will have blood samples collected for the following tests: Complete Blood Count (CBC), Serum Ferritin, and Vitamin D. Based on their hemoglobin levels, participants will be divided into two groups: Group 1 Anemic (Hemoglobin < 10 g/dL), Group 2 Non-Anemic (Hemoglobin ≥ 10 g/dL). The study objectives include:

1. Correlation between iron deficiency anemia and serum vitamin D3 levels. 2) Incidence of vitamin D deficiency in rural pregnant women. 3) Study the effects of vitamin D deficiency on maternal-fetal outcomes. 4) Study the correlation between serum ferritin and vitamin D levels.

DATA ANALYSIS

Data were entered into Microsoft Excel. Mean ± SD were calculated for quantitative data, and percentages and proportions were calculated for categorical data. Generalized estimating equations were used to analyse the association between vitamin D levels and hemoglobin or anemia using SPSS (version 22.0). A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The study was conducted on over 400 pregnant women at the Department of Obstetrics and Gynaecology, Bhagat Phool Singh Girls Medical College, Khanpur, Sonipat, Haryana. The comparison between the anemic and non-anemic groups revealed some notable differences and similarities in various parameters. Both groups were similar in basic characteristics of pregnant women like age, BMI, parity, socioeconomic status, and obstetrics outcome. However, surprisingly the anemia group included more primigravida compared to multigravida. The BMI was also high and normal in both groups; the reason may be the inclusion of the present weight of pregnant women instead of pre-pregnant weight. The rates of antepartum hemorrhage, chorioamnionitis, and gestational diabetes mellitus were similar between the anemic and non-anemic groups. However, significant differences were observed in intrauterine growth retardation (3.5% in the anemic group vs. 1.5% in the non-anemic group) and pre-eclampsia (7.5% vs. 2%, respectively, $p < 0.05$). Normal pregnancy outcomes were more common in the non-anemic group (93.5%) compared to the anemic group (87%), highlighting some of the adverse effects associated with anemia, as detailed in Table 01 of the study.

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Table no 01: Baseline profiles pregnant women factors in both the group

	Group 1 (Anemic)	Group 2 (Non-Anemic)	P value
Age (Mean+ SD)	24.63±4.17	25.12±3.97	0.59
Mean BMI	23.78±3.35	24.303±3.96	0.70
Parity			
Primigravida	52 (26.0%)	28 (14.0%)	0.01
Multigravida	137 (68.5%)	161 (80.5%)	
Grand multiple	11 (5.5%)	11 (5.5%)	
SOCIOECONOMIC STATUS			
Upper	1 (0.5%)	2 (1.0%)	0.60
Upper-Middle	32 (16.0%)	37 (18.5%)	
Lower-Middle	126 (63.0%)	127 (63.5%)	
Upper-Lower	37 (18.5%)	33 (16.5%)	
Lower	4 (2.0%)	1 (0.5%)	
PREGNANCY-ASSOCIATED COMPLICATION	26	13	0.57
Antepartum haemorrhage{abruption)	1 (0.5%)	3 (1.5%)	
Chorioamniotis	0 (0.0%)	1 (0.5%)	
Gestational diabetes mellitus	2 (1%)	2 (1%)	
Hypothyroidism	1 (0.5%)	0	
Intrauterine growth retardation	7 (3.5%)	3 (1.5%)	
Pre-eclampsia	15 (7.5%)	4 (2%)	
Normal	174 (87%)	187 (93.5%)	
Mode of delivery -VD	170(85%)	166(83%)	0.35
LSCS	30(15%)	35(17%)	
Fetal weight (Mean+SD)	2.570+0.39	2.670+0.33	0.01(S)
Postpartum complications	20	0	

Table 02: Comparative presentation of hematological investigation in both groups

	Group 1(Anemic)	Group 2(non-anemic)	P value
HEMOGLOBIN	8.35±1.22	11.31±.96	0.001 (S)
VITAMIN-D	13.42±8.94	18.78±10.66	0.001 (S)
< 20 (deficient)	158 (79%) Mean-9.37±4.71	119 (59.5%) 10.02±4.65	0.001 (S)
20-29.9 (insufficient)	27 (13.5%) 23.52±2.73	40 (20%) 23.73±3.05	
≥ 30 (normal)	15 (7.5%) 34.2±3.07	41 (20.5%) 33.81±4.56	
FERRITIN LEVELS	26.71±34.69	46.42±44.58	0.001(S)
<50ng/ml	175 (87.5%) 17.13±11.5	114 (57%) 22.4±13.23	
50-100ng/ml	21(10.5%) 73.48±23.56	77(38.5%) 62.78±15.81	
>150ng/ml	4 (2%) 205.25±79.12	9 (4.5%) 217±45.62	

Table no-2 shows the comparative presentation of hematological investigations in both groups. The results were significantly different in both groups. Mean vitamin D levels were significantly higher in pregnant women without anemia and the same with ferritin. The analysis of ferritin levels in Table no 2 showed serum ferritin levels i.e<50ng/ml in 57%of non-anemic pregnant women.This showed that with mean Hb of 11.31±0.96.There is a high incidence of depletion of stores in pregnant women. However 12.5% of pregnant women in the anemic group had normal serum ferritin levels. When this table was analyzed for vitamin D levels 277 pregnant women (69.25%)were deficient in vitamin D levels and only 14% pregnant women had normal vitamin D levels.

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Table 03: Vitamin D and Obstetrics Associations

	Vitamin D			
	< 20 (deficient)	**20-29.9 (insufficient)**	**≥ 30 (normal)**	**P value**
OBSTETRICS COMPLICATION	26 (%)	10(%)	3(%)	0.906
MODE OF DELIVERY				
NVD	25(89.9%)	79(91.9%)	51(91.1%)	0.04
LSCS	52(20.2%)	7(8.1%)	5(8.9%)	
FETAL WEIGHT (Mean & Std.dev)	2.60(±0.35)	2.68(±0.35)	2.64(±0.041)	0.209
FERRITIN				
<50	203(78.7%)	62(72.1%)	34(60.7%)	0.03
50-150	49(19.0%)	19(22.1%)	20(35.7%)	
>150	6(2.3%)	5(5.8%)	2(3.6%)	

Table no 3 presents a comparative picture of maternofetal outcomes with different levels of vitamin D In terms of obstetric complications, the rates of antepartum hemorrhage (APH), chorioamnionitis, gestational diabetes mellitus (GDM), and hypothyroidism were similar across the different vitamin D groups, with p-values indicating no significant differences. However, the incidence of obstetrics complications was maximum (%) in vitamin D deficit pregnant women followed by vitamin D insufficient (%) and normal levels of vitamin D. Among the obstetrics complications associated with preeclampsia and IUGR were located in the group of vitamin D deficiency.

Exposure to sunlight did not significantly differ across vitamin D categories, with around 78.7% of those with deficiency, 83.7% with insufficient levels, and 78.6% with normal levels reporting sun exposure, yielding a p-value of 0.58.

Regarding the mode of delivery, a notable difference was observed: 89.9% of those with vitamin D deficiency delivered vaginally, compared to 91.9% in the insufficient group and 91.1% in the normal vitamin D group, with a p-value of 0.04. This suggests a statistically significant association between vitamin D levels and mode of delivery.

Fetal weight did not show significant variation across vitamin D levels, with means of 2.60 kg (±0.35), 2.68 kg (±0.35), and 2.64 kg (±0.041) for the deficiency, insufficient, and normal groups respectively, yielding a p-value of 0.209.

Ferritin levels did show significant differences, with 78.7% of those with vitamin D deficiency having ferritin levels <50 ng/mL, compared to 72.1% in the insufficient group and 60.7% in the normal group, with a p-value of 0.03. This indicates that lower vitamin D levels are associated with a higher prevalence of low ferritin levels. Conversely, a higher percentage of individuals with normal vitamin D levels had ferritin levels in the 50-150 ng/mL range (35.7%) compared to those with deficiency (19%) and insufficiency (22.1%).

DISCUSSION

In our study, conducted among 400 rural pregnant women aged 21 – 35 years. The mean age in both the groups was 24.63± 4.17and 25.12±3.97. Age distribution showed no statistically significant difference between the groups (P = 0.59), which was similar to **Marwaha RK et al (15)** and **Sachan A et al (16)** in pregnant women. The mean BMI is 23.78±3.35 and 24.303±3.96) suggestive of no significance (0.7) in group 1(anemic) and group 2 (non-anemic), two different studies conducted by **Shwetha et.al (17)** and **Tanishq H et.al (18)** on pregnant women found no association between BMI and anemia (Table no 01).

In our study analysing the incidence of vitamin D deficiency and obstetric complications, as depicted in Table 02, we found that these complications were more prevalent among pregnant women with low levels of vitamin D. Studies by **Arora S et al (19). and Flood-Nickols SK et al (20).** have also demonstrated that pregnant women with inadequate vitamin D levels during delivery face a heightened risk of complications such as hypertension, preeclampsia, and preterm labor or delivery, which can increase the likelihood of needing a caesarean section. Additionally, other adverse pregnancy outcomes, including gestational diabetes mellitus and intrauterine growth restriction, have been linked to insufficient vitamin D levels.

In our study the observed clinical outcome (Table 03) between the two different groups. The incidence of postpartum complications like wound infections (1%vs0.0%, P=0.04) and atonic postpartum hemorrhage (8% vs 4%, P=0.04) was significantly higher in the anemic group compared to the non-anemic group. Also the number of pregnant women who developed obstetric complications was double in the anemic group compared to non-anemic pregnant women. So, anemia is a contributing factor that causes obstetrics complications. **Beckert RH et. al (21),Bukhary NBI et al (22),** and **Benson CS et.al (23)**, **Suriyanarayan R et al (24)** reported similar complications.

In our study, sun exposure differed significantly (p=0.01) between anemic (75%) and non-anemic (84.5%) pregnant women (Table 04), aligning with findings by **Sanguina et al (25)**. suggesting lower hemoglobin levels in those with iron-rich diets but inadequate sun exposure. Our study did not establish a

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direct link between sun exposure and vitamin D levels. Both groups showed similar rates of routine antenatal calcium + vitamin D and iron supplementation, with 11.5% of non-anemic women over-supplemented vitamin D compared to 7.5% in the anemic group. Studies by **Tanishq H et al (18).** indicate that correcting vitamin D levels can potentially improve hemoglobin levels in pregnant women.

In our study, Group 1 exhibited significantly lower hemoglobin levels (8.35 ± 1.22 g/dL vs. 11.31 ± 0.96 g/dL, P = 0.001) and a higher prevalence of moderate and severe anemia compared to Group 2. Group 1(Anemic) had lower vitamin D levels (13.42 ± 8.94 ng/ml vs. 18.78 ± 10.66 ng/ml, P = 0.001) and a higher prevalence of vitamin D deficiency (< 20 ng/ml) compared to Group 2(Non-anemic). **TanishqH et.al (18)**, among pregnant women who have anemia, 75% of them had serum vitamin D concentrations lower than 20 ng/ml, while only 52.2% of women in the control group had the same condition. Vitamin D levels were also lower in Group 1 (13.42 ± 8.94 ng/mL vs. 18.78 ± 10.66 ng/mL, P = 0.001), with a higher proportion exhibiting deficiency (< 20 ng/mL) compared to Group 2 (79% vs. 59.5%). Among pregnant women who have anemia, 75% of them had serum vitamin D concentrations lower than 20 ng/ml, while only 52.2% of women in the control group had the same condition. **Suh et al (26)**, 2016 reported that in generally healthy adults, serum 25(OH) D concentrations <20 ng/ml were associated with lower hemoglobin concentrations.

In our study, we observed a significant positive correlation between serum levels of vitamin D3 and serum ferritin. Specifically, out of the 299 (74.8%) pregnant women with serum ferritin levels < 50. 203 (78.7%) had vitamin D3 levels below 20 ng/ml**. Lee et al (27)** reported that healthy Korean women with vitamin D deficiency (25(OH)D < 15 ng/ml) had increased odds ratios for iron deficiency, characterized by low serum ferritin levels (< 12 µg/L) and transferrin saturation (< 16%), as well as iron deficiency anemia (Hb < 13 g/dl and iron deficiency). This underscores the importance of adequate vitamin D levels in maintaining iron balance during pregnancy.

Limitations of the study

The study included the hospital population and being a tertiary centre, it mainly referred high-risk patients, so results can't be extrapolated on the general population. Due to a limited study period, a case-control study was conducted instead of a long study.

CONCLUSION

In our study, highlights the significant impact of anemia and vitamin D deficiency on obstetric outcomes among rural pregnant women aged 21-35 years. Anemic participants showed higher rates of postpartum complications and obstetric issues compared to non-anemic women, underscoring the need for effective management strategies during pregnancy. Low vitamin D levels were prevalent among those with anemia, potentially exacerbating iron deficiency. These findings emphasize the importance of addressing both anemia and vitamin D deficiency early in prenatal care to mitigate adverse pregnancy outcomes and improve maternal and fetal health.

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