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#### Abstract

**Background:** Deficiency of vitamin D is widespread across the globe. Expectant women are one of the most vulnerable groups for vitamin D deficiency (VDD). Even in South India with abundance of sunlight, pregnant women are believed to be at a high risk of this deficiency. The objectives of this study are to assess the prevalence of VDD in antenatal women, associate it with modifiable risk factors and evaluate its correlation with low birth weight. **Methods:** This cross-sectional study was conducted in a tertiary care hospital, in Chennai, in 100 pregnant women in their last trimester on the basis of inclusion and exclusion criteria and their vitamin D and calcium levels were assessed. A detailed history regarding physical activity, diet, and sun exposure were collected and results were analyzed. **Results:** The point prevalence of VDD (serum 25-hydroxyvitamin D (25(OH) D) level <20 ng/mL) among antenatal women in our study is 62%. Univariate analysis revealed that sun exposure and socioeconomic status were the significant factors associated with higher percentage of VDD. Linear regression analysis showed that only sun exposure was a significant predictor for serum 25(OH) D levels. VDD is also associated with increased risk of low-birth-weight babies. **Conclusion:** VDD is highly prevalent among pregnant women in South India leading to adverse health consequences in the mother and offspring. Less physical activity, decreased sun exposure, darker skin complexion, lower socioeconomic status and lack of awareness are the major risk factors associated with VDD in our study population.

**Keywords:** Low birth weight, pregnancy, prevalence, sun exposure, vitamin D deficiency

## Introduction

In the last two decades there has been numerous studies on deficiency of vitamin D during pregnancy from across the world that have divulged several implications in both the mother and the newborn. Unexpectedly, vitamin D deficiency (VDD) has been

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found to be widespread in tropical countries like India where most of its population receive abundant sunlight throughout the year. Chennai, in the southern part of India has a tropical wet and dry climate. Despite the string of benefits that flow from freely available sunlight, the residents of Chennai do lack sufficient exposure to sunlight due to the changing trend in their lifestyle. Growing urbanization, less outdoor activity, increased pollution, darker skin color, and inadequate dietary intake may add up to the onus of this problem.

VDD during pregnancy has many health implications in both antenatal women (increased risk of pre-eclampsia, gestational

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diabetes mellitus) and newborn (low birth weight, neonatal tetany, congenital and infantile rickets)<sup>[1]</sup> and hence it is crucial to prevent VDD during this period. During pregnancy, especially during the third trimester important alterations occur in the metabolism of calcium and phosphate, allowing the accretion of calcium, particularly within the fetal skeleton. It is quite remarkable that during pregnancy the concentration of ionized calcium in the serum of mother remains stable, whereas absorption of calcium from the intestine increases, and extra calcium is mobilized from the maternal skeleton.<sup>[2]</sup> 1,25-Dihydroxyvitamin D (1,25(OH) D) plays a major role in these adaptations, as the levels of free 1,25(OH) D is found to rise relatively in the third trimester. Serum 25-hydroxyvitamin D 25(OH) D in pregnancy is thought to be very important as the fetus is solely dependent on the mother for 25(OH) D and it is found to cross the placenta, partly through the megalin-cubilin endocytotic system.<sup>[3]</sup>

Numerous studies stress on vitamin D influencing a significant number of physiological actions other than bone and muscle health.<sup>[4]</sup> During pregnancy, vitamin D is involved particularly in skeletal development and calcium homeostasis of the foetus.<sup>[5]</sup> Association between VDD in pregnancy and many adverse maternal and foetal outcomes have also been reported. Low levels of vitamin D during pregnancy have important consequences for the newborn including low birth weight.<sup>[6]</sup>

With growing data indicating a connect between pregnancy and VDD, the present study was carried out in a tertiary care hospital of south India to assess the vitamin D status of expectant women in their last trimester and its association with maternal parameters like physical activity, dietary vitamin D and calcium intake, vitamin supplementation, sun exposure, and sunscreen use.

# Subjects and Methods

This cross-sectional study was carried out among 100 pregnant women in their last trimester in the age group of 20–35 years and included primigravida (pregnant for the first time) and multigravida (has been pregnant more than one time) women. Pregnant women with a history of chronic medical illness or intake of drugs interfering with calcium and vitamin D metabolism were excluded. Women with conditions complicating pregnancy like eclampsia, toxaemia, thyroid and parathyroid disorders, and kidney disorders were not recruited in the study.

About 110 subjects were screened in the Obstetrics department of a tertiary care hospital in Chennai by convenience sampling out of which 105 were found to be eligible as per the selection criteria and only 100 consented to participate in the study. The pregnant women were recruited from different areas in Chennai to get a representative sample from different zones of Chennai. Selected participants were explained about the purpose of the research and informed consent was obtained. Institutional Ethical Committee clearance was also obtained. A detailed history regarding personal information, past medical history, history of their physical activity, diet (regarding the dietary calcium and vitamin D intake), and sun exposure were collected by administering the relevant questionnaires. Most of the subjects were antenatal booked cases and received either calcium supplements (500 mg) only or calcium tablets (500 mg) along with 200 IU of vitamin D and we also had women without regular antenatal check-ups who have not received supplements.

Blood samples from the above-mentioned subjects were obtained and tested for 25(OH) D by chemiluminescence immunoassay at the biochemical laboratory of the hospital. Vitamin D sufficiency refers to a circulating 25(OH) D level which is adequate for physiologic needs. The level that represents vitamin D sufficiency is highly controversial. As a minimum 25(OH) D level of 20 ng/mL is essential to influence bone and mineral health,<sup>[7]</sup> we have categorized our subjects as those who are sufficient (>20 ng/mL) and those with hypovitaminosis D (<20 ng/mL). Quantitative estimation of calcium was also done and serum calcium was categorized as optimum levels (>8.4 mg/dL) and hypocalcemia (<8.4 mg/dL).

# Statistical analysis

The data were analyzed using R statistical software version 3.6.3. Continuous variables are presented as mean and standard deviation (SD), or interquartile ranges (IQR). Categorical variables are represented as frequencies and percentages. The serum 25(OH) D levels are specified as the dependent variable. All other variables are considered as independent variables. Normality of the data set was done using Kolmogorov–Smirnov Z test for all the variables entered in the main analyses and based on those suitable statistical tests were selected. Linear regression test was conducted to examine the relative effects of independent variables on serum 25(OH) D levels. A P value <0.05 was considered statistically significant.

# Results

The anthropometric, sociodemographic, and pregnancy profiles of the study population are summarised in Table 1. In our study, 40 mothers were first time pregnant women and 53 were second time and 7 were third pregnancies. The mean age of the study population was  $25.69 \pm 3.56$  years, with most of the subjects in the <30 years age group (92%).

Serum 25(OH) D level categories have varying reference guidelines. Considering 20 ng/mL as the cut-off, most of the subjects had insufficient vitamin D status. Sixty two of the maternal blood samples (n = 100) had 25(OH) D levels <20 ng/mL [Figure 1]. The overall median maternal 25(OH) D levels were 18.15 (IQR 13.26–18.62) [Table 1]. Eight mothers had serum levels <12 ng/mL; 54 women had concentration levels of serum between 12 ng/mL and 20 ng/mL, and 38 had sufficient vitamin D status (concentration levels of serum  $\geq 20$  ng/mL). About 62% of the subjects had an insufficient-deficient vitamin D status, while 38% had a sufficient vitamin D status.

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| Table 1: Baseline demographic and biochemical profile of antenatal women |                                     |                                |                   |  |  |
|--|-------------------------------------|--------------------------------|-------------------|--|--|
|  | Vitamin D insufficiency (<20 ng/mL) | Vitamin D adequacy (>20 ng/mL) | Р                 |  |  |
| Age (years)  | 25.69±3.56                          | 24.8±3.6                       | 0.24*             |  |  |
| BMI (kg/m <sup>2</sup> )   | 26.36±2.7                           | 25.84±3.6                      | 0.45*             |  |  |
| Height (cm)  | 157.22±4.8                          | 156.6±5.3                      | 0.67*             |  |  |
| Calcium (mg/dL)  | 8.9±0.56                            | 8.7±0.58                       | 0.13*             |  |  |
| <8.4 n (%)   | 11 (17.8)                           | 15 (39.4)                      | $0.03^{\dagger}$  |  |  |
| >8.4 n (%)   | 51 (82.8)                           | 23 (61.6)                      |                   |  |  |
| Level of Education n (%)   |                                     |                                | $0.38^{\dagger}$  |  |  |
| Graduate   | 18 (29.3)                           | 16 (50)                        |                   |  |  |
| School level   | 41 (66.1)                           | 21 (65.6)                      |                   |  |  |
| Illiterate   | 3 (4.8)                             | 1 (3.12)                       |                   |  |  |
| Gravida n (%)  |                                     |                                |                   |  |  |
| Primi (first pregnancy)  | 26 (41.9)                           | 14 (36.8)                      | $0.28^{\dagger}$  |  |  |
| G2 (second pregnancy)  | 30 (48.38)                          | 23 (60.5)                      |                   |  |  |
| G3 (third pregnancy)   | 6 (9.6)                             | 1 (2.6)                        |                   |  |  |
| Socioeconomic status $n$ (%)   |                                     |                                |                   |  |  |
| Lower middle   | 32 (100)                            | 0                              | 0.001‡            |  |  |
| Upper middle   | 30 (44.1)                           | 38 (55.9)                      |                   |  |  |
| Awareness about importance of Vit D in health                            |                                     |                                |                   |  |  |
| Yes  | 7 (12.3)                            | 4 (10.6)                       | $0.98^{\dagger}$  |  |  |
| No   | 55 (88.7)                           | 34 (89.4)                      |                   |  |  |
| Sun Exposure   |                                     |                                |                   |  |  |
| <15 min/day  | 45 (72.5)                           | 9 (23.6)                       | $0.001^{\dagger}$ |  |  |
| >15 min/day  | 17 (27.5)                           | 29 (76.4)                      |                   |  |  |
| Physical activity (MET.h/week)   | 111.33±40.33                        | 109.02±43.33                   | 0.79*             |  |  |
| Sedentary mean [IQR]   | 26.66 [2.6-105]                     | 25.86 [8.7-97.4]               | $0.807^{\$}$      |  |  |
| Light mean [IQR]   | 51.82 [4.02-104.8]                  | 48.33 [8.4-102.0]              | 0.501§            |  |  |
| Moderate mean [IQR]  | 32.84 [0.0-89.25]                   | 34.81 [2.4-84.8]               | $0.640^{\$}$      |  |  |
| Consumption of Vitamin D rich foods                                      |                                     |                                | 0.20              |  |  |
| Low  | 14 (22.5)                           | 4 (10.5)                       |                   |  |  |
| Moderate   | 48 (77.5)                           | 34 (89.5)                      |                   |  |  |
| Birth weight   |                                     | × /                            | $0.85^{\dagger}$  |  |  |
| <2.5 kg  | 17 (17.5)                           | 9 (23.6)                       |                   |  |  |
| >2.5 kg  | 45 (72.5)                           | 29 (76.4)                      |                   |  |  |

\*Student t-test; †Chi-square test; ‡Fisher exact test; §Mann Whitney U test



Figure 1: Percent prevalence of vitamin D insufficiency and sufficiency among pregnant women

Among the 62 women in our study who were vitamin D deficient, 43 women had a body mass index (BMI) >25 kg/m<sup>2</sup> with a mean BMI of 26.36  $\pm$  2.7. Around 18% of the women had a diet very low in vitamin D content and 82% of the women had moderate vitamin D and calcium intake status in their diet, but none of them consumed vitamin D rich foods on a regular basis. The mean birthweight of the babies was 2.72 gm (SD: 0.38).

Univariate linear regression analysis of the association between potentially linked factors and VDD–insufficiency status during pregnancy is shown in Table 2.

Linear regression analysis showed that only sun exposure was a significant predictor for serum 25(OH) D levels among the antenatal mothers (estimate ( $\beta$ ) = 5.86, *t* = 6.28, *F*(1, 98) = 39.46, *P* - 0.01). In contrast, all other variables were not significant predictors in the model for serum 25(OH) D levels [Table 2]. The adjusted *R*<sup>2</sup> value was 0.29; therefore, 29% of the variation in vitamin D level would be explained by the model containing sun exposure.

## Discussion

VDD is prevailing as an epidemic worldwide. Increased preponderance of VDD in antenatal women has been reported from different parts of the world, ranging from 45% to 100%<sup>[8]</sup>

| Table 2: Association between 25(OH) D in pregnancy and other variables |          |                |       |                   |  |  |
|--|----------|----------------|-------|-------------------|--|--|
|  | Estimate | Standard Error | t     | <i>P</i> r (> z ) |  |  |
| Age (years)  | -0.19    | 0.15           | -1.29 | 0.19              |  |  |
| BMI (kg/m <sup>2</sup> )   | -0.05    | 0.11           | -0.49 | 0.62              |  |  |
| Height (cm)  | -0.17    | 0.17           | -0.96 | 0.33              |  |  |
| Calcium (mg/dL)  | -1.3     | 0.95           | -1.36 | 0.17              |  |  |
| Education level  | -1.06    | 1.02           | -1.04 | 0.29              |  |  |
| Gravida - G2   | 1.07     | 1.14           | 0.94  | 0.34              |  |  |
| Gravida - G3   | -3.07    | 2.23           | -1.37 | 0.17              |  |  |
| Awareness about importance of vitamin D                                | 0.64     | 1.76           | 0.36  | 0.71              |  |  |
| Sun Exposure <15 min/day   | 5.86     | 0.93           | 6.28  | 0.01              |  |  |
| Birth weight <2.5 kg   | 0.77     | 1.07           | 0.72  | 0.47              |  |  |
| Total score of physical activity (MET.h/week)                          | -0.01    | 0.01           | -0.79 | 0.43              |  |  |
| Food consumption - Low   | 1.20     | 1.43           | 0.84  | 0.40              |  |  |

and in India, it has been found to be 42%-93%.<sup>[9]</sup> A high prevalence of 62% of vitamin D insufficiency was observed among the pregnant women in our study. Likewise, increased prevalence of hypovitaminosis D in pregnant women has already been reported by Jani *et al.*<sup>[10]</sup> (100%), Sachan *et al.*<sup>[9]</sup> (84.3%), Chauhan *et al.* (72.1%), and Dasgupta *et al.* (42%).<sup>[12]</sup>

Although there is no consensus on optimal serum levels of 25(OH) D, majority of experts have defined deficiency of vitamin D as a 25(OH) D level of <20 ng/mL (50 nmol/L).<sup>[13]</sup> According to the Institute of Medicine (IOM) Committee, serum 25(OH) D levels of 16 ng/mL (40 nmol/L) is considered as the median population requirement or estimated average requirement (EAR) and levels of 20 ng/mL (50 nmol/L) is found to be sufficient to meet the requirements of at least 97.5% of the population.<sup>[14]</sup> We found the mean vitamin D concentration of 100 pregnant women in our study to be 18.61 ± 6.8 ng/mL. A study done in New Delhi reported the mean concentration of serum 25(OH) D in 25 pregnant women to be 8.6 ± 4.28 ng/mL.<sup>[15]</sup> In the present study, the age range was 19–36 years and VDD seems to be most common in 25–29 years (51.67%) with mean age of 28.31 ± 3.86 years.

Interestingly BMI was not an independent determinant of 25(OH) D in our study group, though obesity is being associated with low 25(OH) D levels.<sup>[16]</sup> The socioeconomic status of the pregnant women in our study had a high statistically significant association (P = 0.001) with vitamin D levels indicating that prevalence of VDD is more prevalent in lower socioeconomic group than higher socioeconomic group, which is in concurrence with studies done by Sharma *et al.*<sup>[17]</sup> This difference can be attributed to the education of the women, dietary habits, environmental factors, and cost of vitamin D supplements and fortified foods.

In our study, the subjects with hypovitaminosis D had lower 25(OH) D concentrations, probably due to a shorter duration of direct sunlight exposure which was statistically significant (P - 0.001). Furthermore, the surface area of skin exposed to direct sunlight during this period was only 15% as a result of traditional dress (face, forearms, and hands only). Matsuoka *et al.*<sup>[18]</sup> has reported decreased efficiency of vitamin D synthesis in subjects who wore clothing that allowed exposure of only the head and neck region, and hands to sunlight. Under optimal conditions, a minimum of 15 min of daily exposure of the hands and face to the sun is required to produce adequate vitamin D.<sup>[19]</sup>

In our study, 62% of antenatal women had hypovitaminosis D (25(OH) D level <20 ng/mL) though majority of them took a prenatal supplement of vitamin D and consumed some dietary sources of vitamin D daily (1–2 glasses of milk per day and fish at least once or twice a week). Similar observations were found in a study where 73% of the women and 80% of their infants at the time of birth were vitamin D deficient.<sup>[20]</sup> Unfortunately, dietary sources are unlikely to be sufficient, especially for vegetarians, as it is found in sufficient levels in only a few non-vegetarian foods that are regularly consumed like oily fish such as herring, salmon, sardines and mackerel, egg yolks, and liver besides milk products and fortified foods.

An increasing number of studies indicate that some of the harmful effects due to deficiency of Vitamin D is done in utero while the fetus is developing and the damage may not be fully reversed by supplementing Vitamin D after birth.

Pregnancy is a demanding physiological state and there is widespread maternal malnutrition leading to increased prevalence of infants with low birth weight and high maternal mortality. A dietary intake of vitamin D containing natural foods contributes to only 10%–20% of circulating (25(OH) D levels. In our study 22.5% of women with hypovitaminosis D consumed less vitamin D rich foods compared to 10.5% of women with adequate vitamin D levels but less intake of dietary vitamin D. However, the cross-sectional design of this study could not document a causal relationship between dietary intake and VDD.

Pregnant women especially in the last trimester choose activities and physical exercises of low and moderate intensity. It is well known that biological variables like age, number of pregnancies, and trimester significantly affect the level of total physical activity of pregnant women. The results of the present study

#### Conclusion

affirm that active lifestyle among pregnant women is essential and there is a need to raise the overall awareness of benefits of physical activity to inspire women to take up physical exercises during pregnancy for normal development of the foetus. The probable role of physical activity in pregnant women is debatable. Prevailing research shows an association between physical activity levels and vitamin D status in women.[21] While studies in antenatal women are scarce, one study conducted in Germany observed that pregnant women who were physically inactive were two to seven times more likely to have VDD than those who were physically active (>1 h/week) after adjusting for season and independent risk factors.<sup>[22]</sup> VDD during pregnancy predisposes their infants to VDD at birth. Deficiency of Vitamin D in utero leads to retardation of growth and skeletal deformities and is associated with decreased birth weight. In a study conducted by Munmun Yadav et al.,<sup>[23]</sup> 35 (29.1%) antenatal women delivered babies with less birth weight, of which 27 (77.14%) had decrease in Vitamin D levels and barely 8 (22.8%) had adequate levels.

In our study, 74 women delivered babies with normal birth weight (gestation  $\geq$  37 weeks and birthweight  $\geq$  2500 g). 26 (26%) antenatal women delivered babies with low birth weight, of which 17 mothers (65.4%) were vitamin D deficient and only 9 mothers (34.6%) had acceptable levels which was not statistically significant. This is in concordance with a South Indian study on maternal vitamin D status in pregnant women which revealed that above 60% had levels <20 ng/mL around 30-weeks pregnancy and no significant relationship was observed between birth weight and maternal vitamin D status. Likewise, Brooke et al.[24] has stated decreased occurrence of low-birth-weight babies in Asian mothers who were supplemented with vitamin D. Vitamin D is considered an important regulator of fetal growth and many studies have observed an association of vitamin D levels with birth weight. In our study the number is very few to conclude about association.

In summary, poor sun exposure, darker skin complexion, vegetarian food habits, low socioeconomic status, and lack of vitamin D food fortification program in the country explain the high prevalence of VDD in India despite its sunny climate. As 25(OH) D deficiency is the first step in the development of osteomalacia in women, there is a need for more multicentric studies to document this problem in various parts of India.

#### Limitations

The limitations of this study include a limited sample size. In addition, the assessment of sun exposure behavior, dietary intake, and vitamin D supplementation reported by the pregnant women are subject to recall bias; the categorization of sun exposure did not include data on time of day and the proportion of the body area actually exposed to direct sunlight which affect the endogenous synthesis of vitamin D. Nevertheless, the study provides new information on reduced dietary calcium intake and sun exposure behavior in pregnant women in south India. VDD is highly prevalent among pregnant women in South India which leads to adverse health consequences in the mother and the offspring. Less physical activity, decreased sun exposure, skin complexion, low socioeconomic status, and lack of awareness are the major risk factors associated with VDD in our study population. Observational studies of correlation between 25(OH) D deficiency and its risk factors are subject to confounding and do not prove causation. It is therefore essential to integrate screening for VDD in the maternal and child health programs, and initiate intervention like food fortification and supplementation to ensure healthy pregnancy outcomes.

#### **Key Messages**

Vitamin D deficiency (VDD) is highly prevalent in South Indian pregnant women. Recommendations and strategies to integrate screening for VDD in the maternal and child health programs, and initiate interventions like food fortification and supplementation should be developed to ensure healthy pregnancy outcomes.

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### **Conflicts of interest**

There are no conflicts of interest.

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