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# 25 hydroxycholicalciferol levels during Pregnancy in Rural and Urban Population of South India

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ABSTRACT

Maternal 25 dihydroxycholecalciferol is the storage form the vitamin D. It gets activated to 1,25, Dihydroxycholecalciferol (vitamin D3) in the kidneys. Pregnancy increases requirement for the vitamin D3. The 1,25 dihydroxycholecalciferol is produced by the fetal kidneys from maternal sources of 25 dihydroxycholecalciferol. Vitamin D3 is essential for intestinal calcium absorption and bone mineralization in fetus.104 venous blood samples were used to study 25 hydroxycholecalciferol and serum calcium levels. Experimental group involved venous blood samples from 100 ANC between 20-40 years. In the first trimester rural ANC cases had an average 25 hydroxycholecalciferol level of 50.9ng/ml. In Urban participants the average was 31.6nmol/L. Second Trimester ANC in rural sector had an average of 54nmol/L and in urban average was 45nmol/L. In third trimester rural participants had an average of 61nmol/L, urban participants had 28nmol/L. The urban participants in all the three trimesters had 25 hydroxycholecalciferol insufficiency. 25 hydroxycholecalciferol deficiency is higher percentage in first trimester, gradually reduces in second trimester and reaches towards normal limits in third trimester. The placental secretion compensates for the homeostasis and maintenance of normal calcium levels and during third trimester. Active fetal bone mineralization occurs in third trimester.

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## INTRODUCTION

### Vitamin D

Vitamin D also called the sunshine vitamin. Deficiency of vitamin D is now observed globally.

The 25 hydroxycholicalciferol level is the storage form and determines the vitamin D status of a person. 1,25 dihydroxycholecalciferol (calcitriol) is the active form. It has been known for the maintenance of serum calcium and phosphorus metabolism. Serum 25 hydroxycholicalciferol is known for the calcium metabolism and bone health of an individual. It is also being identified with the immune tolerance role in sustaining pregnancy (Pilz *et al.*, 2018). In pregnancy 25 hydroxycholecalciferol plays a crucial role in sustaining the pregnancy. The demand for intestinal calcium uptake increases in pregnancy. Fetal and neonatal vitamin D levels depend on maternal sources (Chen *et al.*, 2020). In conditions of calcium insufficiency or deficiency, 25 hydroxycholecalciferols is known to induce osteoclastic activity on the bones to maintain calcium homeostasis. The demand for cholecalciferol levels is maximum during the first trimester, this gradually is reduced in the second trimester while the levels are maintained to the normal limits during the third trimester (Mahadevan *et al.*, 2012). There is a varied difference in the serum 25 hydroxycholicalciferol levels in rural and urban antenatal cases of Tamil Nadu, South India. The lifestyle, food habits, sunlight exposure, skin pigmentation occupation plays a major role in the Vitamin D levels of the individual. This observational cross-sectional study reveals the serum vitamin D status of Antenatal cases in rural and urban population.

## MATERIALS AND METHODS

The experimental group consist of 90 venous samples were collected from the Ante natal cases,1$^{st}$ to 36$^{th}$ weeks of gestation after getting prior informed consent. There were 45 samples from the rural ANC and 45 samples from the 45 urban ANC. The age group utilized was between 20-40 years of age. The Control group included venous blood samples from 14 women of the same age group between 20-40 years who are non-pregnant. The main occupation was agriculture among the rural participants. The Urban participants belonged to educated working professionals of Information and Technology department, Medical department, Government sector employees and privates working employees. Tamil Nadu in India has tropical climatic conditions with sufficient sunlight exposure. The samples were collected from January – May. Lifestyle, food habits were accessed with questionnaires. The participants were not on supplementation for nutrients except iron and folic acid. Most of the rural and urban participants were unaware of their 25 hydroxycholicalciferol status and its importance. All the participants predominantly had rice based diet. The BMI was observed to be on the higher side.

The study involved ANC cases of rural and urban background. Both educated and uneducated participants were included in the study. After informing the purpose of withdrawing blood and getting the consent. Under aseptic conditions, 5ml of venous blood from antenatal cases were taken during their routine checkup. The blood collected was centrifuged and serum was separated using REMI 250-300 RPM/per minute. The sample was centrifuged for 3 minutes. The serum was separated from the venous blood samples and 25 hydroxy cholecalciferol were estimated using liquid Chromatography with Tandem spectrometry. Serum calcium was also estimated. Tables 1, 2 and 3.

### First Trimester

In the first trimester, the values range between 34.75nmol/L -65.75nmol/L in rural ANC cases average was 50.9nmol/L for rural participants.

The main occupation of these participants was agriculture which involved sufficient sunlight exposure. Their diet chart included dairy and dairy products. The participant was informed about the dietary allowances of minerals like calcium, proteins, fats and other micro nutrients to be taken during pregnancy. Urban ANC population of the same age group between 20-40 years. Working professional in the Information Technology sector participated prevalently (Kumar *et al.*, 2017).

They were aware of the dietary allowances. They also had a sufficient intake of nutrients like calcium, proteins, fats and other micro nutrients. The exposure to direct sunlight was reduced and they had the habit of using sunscreen, mask to avoid direct sunlight exposure. Low 25 hydroxycholicalciferol levels can also become reasons for recurrent spontaneous abortions in urban women. The 25hydroxycholicalciferol levels in the first trimester range from 22nmol/L- 38nmol/L average were 31.6nmol/L in urban participants. (Table 1).

### Second Trimester

The participants in the first trimester were recommended for a supplement. The serum samples of the ANC recommended with supplement was not included so that the exact prevalence of 25 dihydroxycholecalciferol deficiency was analyzed. Hence the cross-sectional study involved new participants for the second trimester. The rural second trimester ANC cases 25 hydroxy cholicalciferol range from 31ng/ml -81ng/ml average was 54ng/ml. In urban ANC cases, 25(OH)D2 range from 6nmol/L — 40nmol/L, the average was 45nmol/L (Table 2).

### Third Trimester

During the third trimester, the 25(OH)D2 in rural ANC ranged from 15nmol/L — 101.25nmol/L with an average of 61nmol/L. The urban ANC during the third trimester had 25 (OH)D2 ranging from 6nmol/L -38nmol/L with an average of 28nmol/L. During the third trimester, the exposure to direct sunlight gradually reduces in urban ANC cases while the fetal demand is high. Hence, the 25(OH)D2 levels in urban ANC observes low (Table 3).

### Control Group

The Control Group included non-pregnant women of the same age group between 20–40 years.7 serum samples from rural and 7 serum samples from urban were collected and used for comparison. The rural women 25(OH)D2 ranging from 45-89nmol/L average was 64. While urban control samples

**Table 1: Comparison between the serum vitamin D values and serum calcium values of the Rural and Urban ANC cases**

| | Rural ANC First trimester | | | | Urban ANC First trimester | | | |
|---|---|---|---|---|---|---|---|---|
| S.No | Pt Age | Gest. Weeks | Serum Calcium | Serum Vitamin D | Pt Age | Gest. Weeks | Serum Calcium | Serum Vitamin D |
| 1 | 24/F | 9 weeks 2 days | 7mg/dl | 56.25 nmol/l | 25 | 6weeks | 9mg.dl | 22 nmol/l |
| 2 | 21/f | 15 weeks | 6.5mg/dl | 59.5 nmol/l | 25 | 9 weeks 3 days | 8.5mg/dl | 28 nmol/l |
| 3 | 28/f | 12 weeks | 10mg/dl | 52 nmol/l | 29 | 8 weeks | 9.2mg/dl | 31 nmol/l |
| 4 | 28/F | 11 weeks 4 days | 8.2mg/dl | 44.25 nmol/l | 27 | 7 weeks | 10mg/dl | 38 nmol/l |
| 5 | 24/F | 12 weeks 4 days | 7mg/dl | 34.75 nmol/l | 22 | 11 weeks | 8.1mg/dl | 28 nmol/l |
| 6 | 25/F | 10 weeks | 7.8mg/dl | 55.5 nmol/l | 24 | 10 weeks | 9.1mg/dl | 30 nmol/l |
| 7 | 25/f | 7 weeks | 8.9mg/dl | 51 nmol/l | 26 | 12 weeks | 9.3mg/dl | 32 nmol/l |
| 8 | 22/F | 11 weeks | 8.3mg/dl | 65.75 nmol/l | 25 | 8 weeks | 8.6mg/dl | 29 nmol/l |
| 9 | 22/F | 9 weeks 5 days | 9.4mg/dl | 54 nmol/l | 23 | 7 weeks 5 days | 8.8mg.dl | 30 nmol/l |
| 10 | 23/F | 8 weeks 2 days | 9.2mg/dl | 36 nmol/l | 21 | 6 weeks 2 days | 9.4mg/dl | 34 nmol/l |
| 11 | 39/F | 12 weeks 6 days | 8.6mg/dl | 40 nmol/l | 25 | 8 weeks | 9.2mg/dl | 32 nmol/l |
| 12 | 27/F | 7 weeks 3 days | 9.2mg/dl | 46 nmol/l | 25 | 9 weeks 3 days | 9.1mg/dl | 33 nmol/l |
| 13 | 22/F | 8 weeks | 8.5mg/dl | 62 nmol/l | 26 | 10 weeks | 9.3ng/ml | 34 nmol/l |
| 14 | 22/F | 9 weeks | 8.8mg/dl | 42 nmol/l | 23 | 12 weeks | 9.5ng/ml | 35 nmol/l |
| 15 | 27/F | 11 weeks | 9.1mg/dl | 64 nmol/l | 26 | 12 weeks | 9.9ng/dl | 38 nmol/l |

recorded 25 (OH)D2 range from 42-68nmol/L, average 57.7nmol/L.

The normal recommended daily allowance is ≥ 50nmol/L in the reproductive age group. The control group itself reports sufficient levels of vitamin D this gradually decreases due to increasing fetal demand of the 25 hydroxycholicalciferol (Table 4).

## DISCUSSION

Vitamin D is considered a steroid hormone more than a nutrient. It is responsible for calcium and phosphate metabolism (Colucci, 2019). It has also proved to be an immunomodulator. Vitamin D (calcitriol, 1,25(OH)2 D3) is considered a true steroid hormone (D hormone), and like glucocorticoids (GCs) and gonadal hormones, may exert several immunomodulatory activities (Veldman *et al.*, 2000). Serum vitamin D deficiency (25(OH) D) causes reduced 1,25(OH)2 D3 availability, which is considered a risk factor for several chronic/inflammatory or autoimmune conditions, including infectious diseases, type 1 diabetes, multiple sclerosis, and especially autoimmune rheumatic diseases (ARD) (Goldsmith, 2015).

Serum 25 hydroxycholicalciferol is the storage form of vitamin D and it's reported to be insufficient limits during gestation (Bhalla *et al.*, 1983). This study reports the serum levels of 25 hydroxycholicalciferol level in rural and urban antenatal cases. The deficient levels of 25 hydroxycholicalciferol can reduce activation levels of 1,25 dihydroxycholecalciferol, which is the active state of the steroid hormone. The availability of the active 1,25(OH)D depends on the substrate 25(OH)D level. The first-trimester 25(OH)D level to reflect maternal vitamin D status in this study (Aziz *et al.*, 2020). The 25 hydroxycholicalciferol undergoes hydroxylation in the liver and later undergoes activation into 1,25 dihydroxycholecalciferol in the kidney (Boonstra *et al.*, 1950).

 

**Table 2: Serum vitamin D and serum calcium levels in the second trimester as a comparison between rural and urban cases**

| | Rural ANC cases Second Trimester | | | | Urban ANC cases Second Trimester | | | |
|---|---|---|---|---|---|---|---|---|
| S. No | Pt Age | Gest. weeks | Serum Calcium | Vit D | Pt Age | Gest. Weeks | Serum Calcium | Vit D |
| 1 | 23/F | 22 weeks | 9.7mg/dl | 55 nmol/l | 25 | 14 weeks | 9mg/dl | 6 nmol/l |
| 2 | 24/F | 24 weeks | 9.9mg/dl | 65 nmol/l | 20 | 18 weeks | 10mg/dl | 38 nmol/l |
| 3 | 24/f | 23 weeks | 9.8mg/dl | 56 nmol/l | 22 | 24 Weeks | 9.2mg/dl | 28 nmol/l |
| 4 | 26/f | 24 weeks | 8mg/dl | 49.75 nmol/l | 30 | 26 Weeks | 8.6mg/dl | 24 nmol/l |
| 5 | 23/f | 28 weeks | 9.8mg/dl | 54.25 nmol/l | 35 | 22 weeks | 9.4mg/dl | 32 nmol/l |
| 6 | 27/f | 4 days | 9mg/dl | 51 nmol/l | 22 | 20 weeks | 9.6mg/dl | 36 nmol/l |
| 7 | 27/f | 18 weeks | 9.8mg/dl | 68 nmol/l | 26 | 20 weeks | 10mg/dl | 40 nmol/l |
| 8 | 27/f | 3 days | 8.3mg/dl | 31 nmol/l | 21 | 22 weeks | 8,2mg/dl | 22 nmol/l |
| 9 | 20/f | 27 weeks | 8.6mg/dl | 42 nmol/l | 25 | 24 weeks | 8.4mg/dl | 26 nmol/l |
| 10 | 24/f | 19 weeks | 9.3mg/dl | 62 nmol/l | 28 | 26 weeks | 9.8mg/dl | 38 nmol/l |
| 11 | 29/f | 20 weeks | 9.8mg/dl | 81.1 nmol/l | 27 | 24 weeks | 9.1mg/dl | 32 nmol/l |
| 12 | 28/F | 5 days | 9.5mg/dl | 44.25 nmol/l | 26 | 20 weeks | 9.4mg/dl | 36 nmol/l |
| 13 | 24/F | 17 weeks | 8.4mg/dl | 34.75 nmol/l | 30 | 18 weeks | 9.6 mg/dl | 38 nmol/l |
| 14 | 25/F | 16 weeks | 8.1mg/dl | 51 nmol/l | 29 | 18 weeks | 8.4mg/dl | 22 nmol/l |
| 15 | 22/F | 14 days | 9.5mg/dl | 65.75 nmol/l | 31 | 24 weeks | 8.1mg/dl | 24 nmol/l |

In gestation, the fetal requirement of 25 hydroxy-cholicalciferol is provided from the maternal blood. Activation of calcidiol to calcitriol occurs in fetal kidneys. Hence, the fetus completely depends on the maternal intestinal absorption. The raw materials needed for the activation needs to be absorbed from the maternal intestinal absorption (Griffin *et al.*, 2000).

Maternal hypovitaminosis in gestation leads to fetal growth rate retardation and low birth weight. According to Nasrin Khalessi et al. in 2015, low serum 25 hydorxy cholicalciferol has led to birth weight retardation and improper skeletal malformation. The Fetal head circumference was ≤33cm (Griffin *et al.*, 2001).

The study further states that the prevalence of maternal 25 hydroxy cholecalciferol deficiency was 47% in Iran (Ross *et al.*, 2011).

According to Khalessi *et al.* (2015), Depending on mothers 25- (OH)-vit D level, all mothers were categorized as deficient (< 25 nmol/L), insufficient (25-50 nmol/L), normal was ≥50nmol/L above 250 nmol/L was taken to be toxic (Khalessi *et al.*, 2015). The participants were educated about the 25 hydorxycholicalciferol status and calcium status. The participants with deficiency limits were supplemented by the Obstetrician. The participants of the first, second and third trimester were new participants and the same participants were not involved (Cutolo *et al.*, 2014).

There was 40% of the rural ANC in the insufficient limits, that is, 25 (OH) levels ≤ 50nmol/L, while 60% of the rural ANC was within normal limits in the first trimester. All the urban ANC cases were in the insufficient limits during the first trimester (100% insufficient) (Aziz *et al.*, 2020).

During the second trimester, the rural ANC had 33% insufficient levels and 67% were within normal limits (above 50nmol/L). All the urban ANC who participated in the study were in insufficient limits. In the third Trimester, 27% of rural ANC were in the insufficient stage and 73% were within normal limits (above 50nmol/L). The urban ANC participants were reported with insufficient limits.

Control group in rural and urban non pregnant women in the same age group (20-40 years) also had 25 hydroxycholicalciferol insufficiency. In rural, it was 14% insufficiency and 28.5% insufficiency in urban non pregnant women.

The study concludes that rural pregnant women with agriculture as the occupation had serum 25 hydroxycholicalciferol levels above 50nmol/L. The insufficiency percentage reduced gradually from the first trimester till the third trimester. While the Urban population had reduced sunlight exposure and reduced vitamin D fortified foods were in the insufficient status (Kumar *et al.*, 2017).

**Table 3: Serum25 hydroxycholicalciferol and serum calcium in the third trimester**

| | Rural ANC Cases | | | | Urban ANC Cases | | | |
|---|---|---|---|---|---|---|---|---|
| S. No | Pt age | Gest. weeks | Serum Calcium | Vit D | Pt Age | Gest. weeks | Serum Calcium | Vit D |
| 1 | 28/F | 35weeks 3 days | 8.9mg/dl | 15.25nmol/l | 30 | 26 weeks | 8.6mg/dl | 24 nmol/l |
| 2 | 20/f | 32 weeks | 9mg/dl | 59.9 nmol/l | 28 | 26 weeks | 9.8mg/dl | 38 nmol/l |
| 3 | 28/f | 29 weeks 6 days | 7.8mg/dl | 29 nmol/l | 28 | 26 weeks | 8.8mg/dl | 28 nmol/l |
| 4 | 22/F | 31 weeks 3 weeks | 10.4mg/dl | 42 nmol/l | 24 | 24 weeks | 9.2mg/dl | 24 nmol/l |
| 5 | 27/F | 30 weeks 3 days | 11mg/dl | 64 nmol/l | 24 | 24 weeks | 9.3mg/dl | 26 nmol/l |
| 6 | 20/F | 34 weeks | 8.7mg/dl | 72 nmol/l | 28 | 26 weeks | 9.8mg/dl | 38 nmol/l |
| 7 | 26/F | 34 weeks | 8.2mg/dl | 63.25nmol/l | 30 | 26 weeks | 8.6mg/dl | 24 nmol/ |
| 8 | 21/F | 30 weeks | 7.3mg/dl | 86.5 nmol/l | 26 | 30 weeks | 9.9ng/dl | 38 nmol/l |
| 9 | 26/F | 34 weeks | 9.4mg/dl | 56.25nmol/l | 24 | 32 weeks | 8.8ng/dl | 28 nmol/l |
| 10 | 20/F | 33 weeks | 10.5mg/dl | 72.75 nmol/l | 25 | 34 weeks | 8.7ng/dl | 26 nmol/l |
| 11 | 39/F | 34 weeks 6 day | 11.2mg/dl | 101.25 nmol/l | 26 | 36 weeks | 9.2ng/dl | 32 nmol/l |
| 12 | 22/F | 33 weeks 4 days | 9.5mg | 43.5 nmol/l | 28 | 33 weeks | 8.8ng/dl | 24 nmol/l |
| 13 | 24/F | 35 weeks | 9.3mg/dl | 55 nmol/l | 25 | 31 weeks | 9mg/dl | 6 nmol/l |
| 14 | 26/F | 32weeks 4 days | 8.2mg/dl | 78 nmol/l | 25 | 29 weeks | 9.1mg/dl | 33 nmol/l |
| 15 | 28/F | 34weeks | 9.4mg/dl | 80 nmol/l | 26 | 34 weeks | 9.3ng/ml | 34 nmol/l |

**Table 4: Non pregnant women of the same age group 20-40years in rural and urban conditions**

| Control Group | | | | | | |
|---|---|---|---|---|---|---|
| | Non pregnant rural women (20-40years) | | | Non pregnant urban women (20-40y) | | |
| S. No | Age | Calcium | Cholecalciferol | Age | Calcium | Cholecalciferol |
| 1 | 27 | 9.8mg/dl | 45.45nmol/L | 40 | 10.3mg/dl | 45nmol/L |
| 2 | 35 | 9.3mg/dl | 58.78nmol/L | 32 | 11.4mg/dl | 68nmol/L |
| 3 | 42 | 8.5mg/dl | 68.45nmol/L | 37 | 12.1mg/dl | 76nmol/L |
| 4 | 33 | 8.8mg/dl | 54.93nmol/L | 26 | 11.8mg/dl | 62nmol/L |
| 5 | 48 | 10.3mg/dl | 89.7nmol/L | 23 | 8.4mg/dl | 56nmol/L |
| 6 | 18 | 9.6mg/dl | 70.2nmol/L | 27 | 9.2mg/dl | 55nmol/L |
| 7 | 36 | 8.1mg/dl | 60.78nmol/L | 31 | 11.3mg/dl | 42nmol/L |

This study denotes that urban ANC should have sufficient sunlight exposure and avoid using sunscreens. Vitamin D fortified foods should be included in the diet. The urban participants were from information technology related occupation hence their working hours reduced the sunlight exposure (Wen *et al.*, 2020). The urban control group had 28.5% insufficient cases while rural non pregnant women had 14% 25 hydroxycholicalciferol insufficiency.

25 hydroxycholicalciferol levels play a major role in forming active 1,25 dihydroxy cholecalciferol levels as well as it is responsible for the maintenance of serum calcium levels along with the parathyroid hormone. The total normal fetal skeleton has accreted about 30 g of calcium by the end of gestation, but about 80% of the accretion occurs rapidly during the third trimester. During the third trimester daily accretion level was 250-300 mcg (Kovacs, 2001). Maternal calcium sources are the major sources. Maternal renal calcium excretion is reduced. Excretion of calcium increases with elevated levels of parathyroid hormone.

Fetal demand for calcium during the third trimester for fetal bone mineralization is maintained (Agudelo-Zapata *et al.*, 2018).

By the maternal intestinal increased absorption or compensatory maternal bone demineralization, calcium resources are provided for the fetus (Maggi *et al.*, 2005). Maternal 25 hydroxycholicalciferol insufficiency was 40% during the first trimester, 33% during the second trimester and 26% during the third trimester. This indicates that the maternal compensation occurs during the third trimester to maintain normal calcium and sufficient vitamin D status (Nancy and Erlebacher, 2014). Acute insufficiency levels may lead to fetal birth weight reduction (Chen *et al.*, 2007).

## CONCLUSIONS

The observational cross-sectional study was performed to determine the vitamin D status during gestation. Maternal serum 25 hydroxycholicalciferol level determines the vitamin D status of the person. It is insufficient during the first trimester of gestation. It has been observed to be very low in the urban ANC than the rural ANC. The 25 hydroxycholicalciferol levels gradually increase in the second trimester and reach sufficient or desirable limits in the third trimester. The Urban ANC had prevalent vitamin D insufficiency when compared to the rural ANC. There was considerable sunlight exposure for the rural participants, while there was very less sunlight exposure for the urban participants despite a nutritious diet. Further studies are needed to develop Clinical guidelines to determine the desirable limits of serum vitamin D levels during pregnancy in the Indian population.


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### Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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