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## Original Research Article

## Calcium and vitamin D supplementation to pregnant women in urban maternity centre

Amrita Pramanik<sup>1</sup>, K Kalaivani<sup>1</sup>, Prema Ramachandran<sup>1,\*</sup><sup>1</sup>Dept. of Public Health Nutrition, Nutrition Foundation of India, New Delhi, India

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## ABSTRACT

**Background:** Prevalence of vitamin D deficiency in pregnant women in India is high. There have been case reports from India of neonatal tetany and vitamin D deficiency in breast-fed infants. India initiated the calcium and vitamin D supplementation programmes for pregnant women a decade ago. There is a need to assess compliance with calcium and vitamin D supplementation and impact of supplements on vitamin D levels in pregnant women.

**Materials and Methods:** Women attending antenatal clinic in an urban maternity centre who fulfilled the eligibility criteria and were willing to participate in the study were enrolled. They were given one month's supply of supplements, and form for recording compliance and side effects and were followed up every month. Blood samples were drawn at enrolment and after three months of supplementation and plasma vitamin D levels were estimated.

**Results:** Two third of the women had vitamin D level below 20 ng/ml at enrolment. There was excellent compliance with supplementation; women who were followed up consumed 94% of the tablets provided. None of the women had clinical signs suggestive of vitamin D deficiency or any major obstetric problems; their offsprings were healthy. A third of the pregnant women continued to have low vitamin D levels after three months supplementation.

**Conclusion:** The current dose of supplements appears to be inadequate to correct the vitamin D levels in deficient individuals. There is a need to undertake studies to find out the optimal dose of vitamin D supplementation which corrects vitamin D deficiency as assessed by maternal vitamin D levels.

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## 1. Introduction

In the early part of the last century, osteomalacia in women and rickets in young children were well-recognized clinical problems.<sup>1,2</sup> Over the second half of the last century, osteomalacia was no longer seen and there was a decline in rickets in children. With the reduction in the clinical cases of vitamin D (vit D) deficiency, the interest in studies on vit D deficiency waned. Availability of 25(OH)D assay to assess blood levels of vitamin D

made it possible to diagnose asymptomatic vit D deficiency. Studies from India documented high prevalence of vit D deficiency (vit D level below 20ng/ml) across all age, sex, and socio-economic groups<sup>3-5</sup> in pregnant women and their offspring at birth.<sup>3-7</sup> Studies mostly from developed countries reported an association between low vit D levels in pregnant women and pregnancy-induced hypertension, gestational diabetes, poor intrauterine growth, and preterm births; but these associations might not be causally related to vit D deficiency.<sup>8-13</sup> During the last two decades, there have been case reports from India on neonatal tetany, vit D deficiency in breast-fed infants, and clinical and radiological rickets in children of all ages.<sup>14-16</sup> Globally and in India

\* Corresponding author.

E-mail address: [premaramachandran@gmail.com](mailto:premaramachandran@gmail.com) (P. Ramachandran).

interventions to prevent vit D deficiency across population groups through food fortification and management of vit D deficiency in pregnancy through Calcium (Ca) and vit D supplementation have been given high priority.

Following the reports of neonatal tetany in Indian immigrants in the UK, studies were taken up to assess the impact of supplementation with 1000 IU of vit D in the third trimester; supplementation resulted in an improvement in maternal vit D levels and a reduction in neonatal tetany.<sup>17</sup> Subsequently, there had been several publications reporting the impact of vit D supplementation to pregnant women between 600 IU/day to 4000 IU/day during the second and third trimester.<sup>18</sup> Cochrane systematic reviews and other meta-analyses indicate that vit D supplementation during pregnancy increases serum 25-OH vit D at term and improves neonatal vit D levels. However, there is no unequivocal evidence indicating that vit D supplementation at low dose of 400-600 IU/day or in higher doses up to 4000 IU/day had a beneficial impact on maternal health.<sup>19–22</sup>

India's National Guidelines for "Calcium Supplementation During Pregnancy and Lactation" envisage supplementation with tablets containing elemental calcium 500mg (as calcium carbonate) and 250 IU vitamin D (as cholecalciferol); two tablets were to be taken by pregnant women twice daily after a meal from the second trimester of pregnancy until six months postpartum.<sup>23</sup> There are very few publications on compliance with Ca and vit D supplementation and impact of supplements on vit D levels in pregnant women. A research study was taken up to assess compliance with Ca and vit D supplementation under research conditions and its impact on maternal vit D levels.

## 2. Materials and Methods

The study design is given in Figure 1. Women attending the antenatal clinic during the second trimester were informed about the study and those willing to participate in the study were screened for inclusion criteria. Those who fulfilled the inclusion criteria (Figure 1) were given the study information sheet and the proposed study was explained to them in detail. They were requested to consult with their family and if willing to participate in the study return one week later. Written consent was obtained prior to enrolment. At enrolment information on the socio-demographic profile of their family, the obstetric and nutritional profile of the woman were obtained. Height, weight and blood pressure were measured and obstetric examination findings were recorded; 2 ml of blood was drawn, processed, and stored until analysis.

In Delhi, both iron folic acid (IFA) and Ca and vit D (Ca and vit D) supplementation to pregnant women are being provided in antenatal clinics in hospitals. The guidelines provided by intensified national iron plus initiatives (I-NIPI) envisage that all pregnant women were to be screened

for anaemia and anaemic women were to be given 2 tablets of iron and folic acid (60mg of elemental iron and 500µg of folic acid) together after a meal. Ca and vit D supplementation guidelines state 2 tablets of Ca and vit D should be taken after two separate meals and IFA and Ca and vit D tablets should not be taken together. Most pregnant women come from families with a habitual three-meal pattern and by following these guidelines women should be able to take both 2 tablets of IFA and 2 tablets of Ca and vit D tablets every day.

The antenatal clinic provided IFA and Ca and vit D supplements to women attending the antenatal clinic as and when they were available. The supply of Ca and vit D supplements was more often disrupted as compared to the iron and folic acid supplies. Ca and vit D tablets were more expensive as compared to IFA tablets; when clinicians prescribed the tablets because they were not available in the hospital, many women were unable to purchase Ca and vit D tablets. In view of this the research team provided the Ca and vit D supplements (containing elemental Ca 500 mg and 250 IU vit D) to all women who were participating in the study, and they were requested to take one tablet twice a day with breakfast and dinner. These women were given a form for recording compliance and side effects, and were requested to bring the tablet strip(s) from which they had taken the tablets at the next follow-up.

Follow-up was done once a month. At each follow-up weight and BP were measured; obstetric examination findings and compliance with supplementation were recorded. At 3 month follow up 2 ml of blood was drawn for vit D estimation. Blood sample was processed and the separated plasma was stored at -20°C till analysis. An attempt was made to provide supplements till 38 weeks and also collect information on delivery and birthweight of the offspring.

Enrolment began in mid-2019. Till March 2020 147 women were enrolled for the study and given the supplements; 64 women had completed three-month follow-up and had their follow up blood samples collected. On 24th March 2020, India imposed a strict lockdown to delay the spread of SARS CoV 2 pandemic in the country. Over the next five months, neither the research team nor the study women could move about freely and reach hospitals for scheduled visits; therefore, the study was terminated. Data at enrolment and follow-up, collected till March 24th, 2020 were analysed to obtain some idea about compliance with the Ca and vit D supplementation under research conditions, and the impact of supplementation on maternal vit D levels.

Assays for vitamin D3 were carried out in paired samples (at enrolment and after 3 months of supplementation) from 64 women in whom both samples were available. Vitamin D3 assay was done in a NABL-accredited laboratory in New Delhi in Cobas 6000 using electro chemo luminescence immunoassay.

Pregnant women attending antenatal clinic in the second trimester  
Provide Study Information Sheet and discuss the rationale of the study  
Ascertain whether they are willing to participate in the study.  
**If yes** screen for inclusion criteria

**Inclusion criteria:**

Apparently healthy pregnant women, living near hospital and willing to come for follow-up;  
No history of systemic or obstetric problems;  
Willing to provide information every fortnight on regularity of tablet consumption and side-effects  
Willing to provide blood sample at enrolment, 12 weeks later and  
Continue taking the supplements till delivery;  
Share information on course and outcome of pregnancy and birthweight;

**At enrolment:**

Record socio-demographic profile of the family; obstetric profile of the women  
Record height, weight, blood pressure and obstetric examination findings for the pregnant woman;  
Collect intravenous blood samples for estimation of vit D  
Blood sample processed and stored at -20° C till analysis;  
Provide supplements for fortnight  
Request her to bring the tablet strips from which she had taken the tablets at next follow up visit

**Monthly follow-up**

At each follow-up  
Record weight, blood pressure and obstetric examination findings;  
Record information on supplement consumed and side effects with supplement;  
Supply supplements for the next month;  
Request her to bring the tablet strip(s) from which she had taken the tablets at next follow-up  
Provide forms for recording compliance and side effects.

**Three month follow up**

In addition to above collect blood samples for estimation of vit D

**At delivery:**

Collect information on course and outcome of pregnancy and  
Birthweight sex and health status of the offspring

**Fig. 1:** Study design

The study was approved by the Ethics Committee of Nutrition Foundation of India. Permission to carry out the study in the maternity centre was obtained from the Dept of Health of the National Capital Region.

### 2.1. Sample size

There was no data on the impact on plasma vit D after supplementation of 1000 mg of Ca and 500 IU of vit D in pregnant women, based on which the sample size could be calculated. There are substantial seasonal variations in vit D levels. To adjust for these variations, it was decided to enrol 100 pregnant women per season for each of the four seasons.

## 3. Results

### 3.1. Socio-demographic, obstetric, and nutrition profile

The socio-demographic profile of the pregnant women is given in Table 1. The majority of women were from nuclear families. Nearly half the families had 4-8 members. About 1/6<sup>th</sup> of the women had no schooling; about half of them had school education, and about one-third had a college education. Over half of the husbands had a school education and over 40% had a college education. Over 95% of the women were homemakers. The majority of the men were working as semi-skilled or skilled workers. (Table 1).

**Table 1:** Socio-demographic profile of women (147)

<b>Type of family %</b>	
Joint	51.0%
Nuclear	49.0%
<b>Family size %</b>	
≤ 3	38.8%
4-8	50.3%
>8	10.9%
<b>Woman's education %</b>	
No schooling	17.7
Had schooling	46.3
College	36.1
<b>Husband's education %</b>	
No schooling	5.4
Had schooling	51.7
College	42.9
<b>Woman's work status %</b>	
Home maker	95.9
Working outside	4.1
<b>Husband's work status %</b>	
Unskilled	5.4
Semi-skilled	47.6
Skilled	42.9
Not working	2.0

The obstetric profile of the women is given in Table 2. The majority of women were in their twenties and were having their first or second child. The gestational age at enrolment was 16 weeks. The nutritional status of women at

enrolment is shown in Table 3. The mean height of women was 151.6 cm and the mean weight was 52.2 kg; their mean Hb was 10.1g/dL.

**Table 2:** Obstetric profile of the women (147)

Age (yr)	25.2 ±3.8.0
Age at marriage (yr)	20.6±3.33
Gravida	2.0±0.98
Para	0.8±0.82
Previous Abortions	0.3±0.53
Live births	0.8±0.82
Gestational age at enrolment	16.1±2.66

**Table 3:** Nutritional status at enrolment (147 women)

Height (cm)	151.6±4.75
Weight (kg)	52.2±9.2
Hb g/dL	10.1±1.34

There were no significant differences in the socio-demographic, obstetric, and nutritional profile of the 147 women who were enrolled for the study and the 64 women who had come for follow-up for 3 months. It is, therefore, likely that the finding about the vitamin D status in the 64 women who had the supplement for 3 months may provide useful preliminary information on compliance with Ca and vit D supplementation and the impact of supplementation on vit D levels in pregnant women.

### 3.2. Compliance with supplementation

Analysis of data on compliance with supplementation and side effects from 64 women who had completed 3 months of supplementation, showed that none of these women complained of troublesome side effects; some women stated that they occasionally felt fullness in the stomach after food. They were reassured by the research team and requested to continue the supplements; all of them continued taking the supplements. With an assured regular supply of the supplements and supportive supervision of the research team, these 64 women consumed 94% of the tablets to be consumed during the first 90 days of supplementation.

### 3.3. Vitamin D levels at enrolment and follow up

At enrolment mean vitamin D3 levels were 19.6 ng/ml and 61% of the women were having vit D levels below 20 ng/ml suggestive of vit D deficiency. After three months of Ca and vit D supplementation with over 90% compliance, the mean vit D levels increased to 29.5 ng/ml; prevalence of vit D levels below 20 ng/ml came down to 34% (Table 4).

Among those who were deficient (<20ng/ml) at enrolment; 46% improved and became non-deficient, but 54% remained deficient. Among women who did not have vit D deficiency at enrolment, 16% became deficient at follow-up (Table 5).

**Table 4:** Plasma vitamin D levels at enrolment and three months follow up

	<b>Enrolment</b>	<b>Three month follow up</b>	<b>Test of significance</b>
Vit D levels ng/ml	19.6±12.81 (64)	29.5±16.44 (64)	One tailed Paired t test (T≥ t) = 0.0001
Vit D <20 ng/ml prevalence (%)	60.9	34.4	Chi square test 0.003

**Table 5:** Changes in prevalence of vitamin D deficiency between enrolment and three months follow up

<b>Vit D at enrolment</b>	<b>Three month follow up &lt;20ng/ml</b>	<b>Three month follow up ≥ 20 ng/ml</b>
<20ng/ml (39)	46.2%	53.8%
≥ 20ng /ml (25)	16%	84%

Chi square test P value 0.01

#### 4. Discussion

Globally vit D levels below 20ng/ml suggestive of vit D deficiency is seen in majority of pregnant women. Taking cognisance of the reported high prevalence of vit D deficiency in pregnancy and the potential adverse health consequences of vit D deficiency on the mother-child dyad, most countries have initiated vit D supplementation programmes for pregnant women. Ongoing research efforts aim at clearly documenting:

1. The maternal vit D levels indicative of deficiency,
2. Clinical criteria for vit D deficiency in pregnant women,
3. Association if any between low maternal vit D levels and adverse maternal and neonatal health outcomes; and
4. Optimal dose of vit D supplementation in pregnancy to improve vit D levels and bring about reduction in the adverse health consequence, if any, seen in association with vit D deficiency.

It had long been believed that in sun-drenched India de novo synthesis in the skin following sun exposure is the major source of vit D. In the last three decades studies from different parts of India with population groups with varying levels of exposure to the sun, had shown that the majority of Indians have circulating vit D3 levels below <20ng/ml. Part of this might be attributable to an increasing tendency for a sedentary lifestyle, limited exposure to the sun, darker skin colour, and air pollution. Given the widespread prevalence of low plasma vit D levels, increasing attention is being paid to dietary sources of vit D and the role of food fortification in improving vit D levels in Indians. Dietary vit D can be:

1. Vitamin D2 (ergocalciferol) from sources such as plants, mushrooms and yeast, and
2. Vitamin D3 (cholecalciferol) from animal foods such as fish and eggs.

Indian diets are predominantly plant based. Recently published Indian Food composition data show that cereals and pulses, do contain vitamin D2. Supplementation with vitamin D2 was found to increase the level of 24, 25(OH)D3 but in the absence of studies measuring the vitamin D2 metabolites along with PTH and calcium homeostasis markers in Indian settings, it is difficult to ascertain the contribution of dietary sources of vitamin D to the overall vitamin D status. The ICMR-NIN Expert Group recommended the Estimated Average Requirement of vit D of 400 IU/day and Recommended Dietary Allowance of 800 IU/day for men, women, and pregnant women.<sup>24</sup>

There are substantial differences in recommendations regarding vit D levels below which persons may be considered vit D deficient. Meta-analysis of global data and Cochrane reviews suggest that the current evidence base does not allow definite conclusions to be drawn regarding the circulating concentration of 25(OH)-vitamin D during pregnancy suggestive of vit D deficiency.<sup>19-21</sup> In the absence of clear-cut data, the current consensus is to use vit D levels below 20ng/ml as indicative of vit D deficiency in pregnancy.

The limited data from the present study showed that none of the pregnant women with vit D levels below 20 ng/ml at enrolment had any clinical signs suggestive of vit D deficiency, did not have any major obstetric problems and their neonates were healthy. The number of persons investigated was small as the study had to be terminated because of the COVID-19 pandemic-related lockdown in March 2020; therefore, definite conclusions cannot be drawn from the study.

Global efforts are underway to document the clinical criteria for diagnosis of vit D deficiency in pregnancy and adverse maternal and neonatal consequences associated with maternal vit D deficiency. All the available data indicate that clinical manifestations of maternal vit D deficiency are rare. There had been reports that low maternal vit D levels were associated with a higher prevalence of a wide variety of obstetric problems such as pregnancy-induced hypertension, gestational diabetes mellitus, recurrent pregnancy loss, higher caesarian section, postpartum hemorrhage, postpartum depression.<sup>8-13</sup> There is no unambiguous evidence causally linking vit D deficiency to these obstetric problems.

Publications from India have shown that:

1. Majority of the pregnant women had vit D levels below 20ng/ml during pregnancy but none had reported clinical vit D deficiency in pregnant women;
2. The neonates born to women with low maternal vit D levels had low vit D levels but were healthy.<sup>6,7</sup>

There had been case reports of neonatal tetany and rickets in breast-fed infants.<sup>14–16</sup>

Given the reported high prevalence of vit D deficiency in pregnancy (vit D levels <20ng/ml) both in developed and developing countries and the potential adverse consequences of the deficiency on maternal and child health, most countries recommend Ca and vit D supplementation during pregnancy. There is however wide divergence in the dose of supplement provided. Indian guidelines recommend 500 IU.<sup>23</sup> Most developed countries provide vit D supplements ranging from 600–2000 IU/day,<sup>19,20</sup> some advocate doses as high as 4000 IU/day.<sup>18</sup> Lower dose is advocated by those who believe that there is no documented increase in requirement for vit D during pregnancy and providing higher doses of the fat-soluble vitamin may have adverse consequences on mother-child dyad.<sup>20</sup> Proponents of the higher dose argue that the lower doses do not raise maternal vit D to normal levels and may not be effective in preventing the relatively rare adverse consequences of vit D deficiency as well as preventing or reducing obstetric problems such as higher rates of pregnancy-induced hypertension, gestational diabetes, and preterm births associated with maternal vit D deficiency.<sup>18,22</sup>

There had been reports from randomized clinical trials suggesting that the prevalence of some of these problems was lower in women who received high dose (4000 IU) vitamin D supplementation daily.<sup>18</sup> Such high dose supplements had been reported to reduce the prevalence of low birthweight and preterm delivery.<sup>18,21</sup> Cochrane review suggests that there is no clear documentation either of a higher prevalence of obstetric problems in women with low vitamin D levels or reduction in these problems in women who had received vitamin D supplementation.<sup>19,20</sup>

An earlier Delhi study on Ca (500mg) and vit D (250 IU) /day for twelve weeks showed that despite excellent compliance with the supplementation, the improvement in mean vit D levels and reduction in prevalence of vit D deficiency was small (though statistically significant).<sup>25</sup> In the present study, at enrolment nearly two-thirds of the pregnant women had plasma vit D levels below 20 ng/ml. Despite over 90% compliance with the supplementation for 3 months, at the dosage recommended in the National Guidelines on Ca and vitamin D supplementation, one-third of the women continued to have vit D levels below 20 ng/ml. Analysis of data on changes in the prevalence of vit D deficiency after 3 months of supplementation showed that in women who were deficient (<20ng/ml) at enrolment; 54% remained deficient. Obviously, the dose provided was inadequate to correct the deficiency in all deficient women. There is a need to undertake studies to define optimum dose of Ca and Vit D supplementation that corrects vit D deficiency in pregnant women.

There are many clinicians who do not advocate calcium and vitamin D supplementation in pregnant women because:

1. Despite high prevalence of vit D level below 20ng/ml in pregnant women, neonatal tetany is rare in India.
2. Cochrane review suggests that there is no clear documentation either of a higher prevalence of obstetric problems in women with low vit D levels or reduction in these problems in women who had received vit D supplementation.<sup>19,20</sup>
3. Calcium and vit D tablets are expensive; supplementation programme for over 25 million pregnant women year after year requires logistic and manpower support.

These clinicians suggest that decisions about the Ca and vit D supplementation programme for pregnant women can be taken after there is clear documentation of health problems associated with vit D deficiency in pregnant women and the benefits of the supplementation on these are documented in India.

Data from the present study indicate that among women who did not have vit D deficiency at enrolment, 84% remained normal after three months. It is important to find out whether majority of women who had normal vit D levels in early pregnancy continue to have normal levels of vit D in the absence of vit D supplementation. In view of the potential long term adverse health consequences of sustained vit D deficiency, India had embarked on voluntary fortification of milk and oil with vit D. Because of the relatively centralized production and increasing use of packaged edible oil in India, fortified oil may reach a larger proportion of the population. It is important to document the impact of oil fortification on plasma vit D levels. As a measure of abundant caution, the current programme for Ca and vit D supplementation in pregnant women may have to be continued, until research studies clearly show that oil fortification results in a substantial and sustained reduction in vit D levels below 20ng/ml, and that there was no deterioration in the vit D status during pregnancy.

#### 4.1. Strength of the study

The study documents that if supplements are provided regularly and there is supportive supervision, compliance with Ca and vitamin D supplementation is high because of the absence of any side effects. The study also documents that the current dosage of vitamin D is inadequate to correct low vitamin D levels in all pregnant women despite very high compliance with supplementation.

#### 5. Limitation of the Study

The sample size was small due to termination of the study in the first year due to COVID 19 pandemic and therefore definite conclusions cannot be drawn from the study.

## 6. Conclusion

Data from the present small, truncated study confirms that nearly 2/3<sup>rd</sup> of pregnant women have vit D levels below 20ng/ml; none of them had clinical signs suggestive of vit D deficiency or any major obstetric problems. The research team provided the Ca and vit D supplements (containing elemental Ca 500 mg and 250 IU vit D) to all women who were participating in the study and they were requested to take one tablet twice a day with breakfast and dinner.

There was excellent compliance with supplementation; women who were followed up consumed 94% of the tablets provided. None of women who were followed up had clinical signs suggestive of vitamin D deficiency or any major obstetric problems; their offsprings were healthy. A third of the pregnant women continued to have low vit D levels after three months of supplementation the current dose of the supplements appears to be inadequate to correct the vit D levels in deficient individuals and sustain the normal vit D levels. There is a need to undertake studies to find out the optimal dose of vit D supplementation which results in correction of the vit D deficiency as assessed by maternal vit D levels.

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## 8. Conflict of Interest

The authors do not have any conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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### Author biography

**Amrita Pramanik**, Research Scholar

**K Kalaivani**, Deputy Director

**Prema Ramachandran**, Director

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