Effect of vitamin D status in pregnancy on anthropometric measures in the offspring: A prospective observational study

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Abstract

Aim: The aim of the present study was to determine the vitamin D status of pregnant mothers and its effect on anthropometric measures in the offspring.

Methods: A prospective observational study was conducted in the Department of Obstetrics & Gynaecology and Department of Pediatrics, Narayan Medical College and Hospital, Sasaram, Rohtas, Bihar, India, between February 2019 to January 2020. Convenient sampling technique was employed and all mothers attending the antenatal clinic in their 3rd trimester, after taking the proper informed consent were enrolled in this study. Gestational age was calculated using mother’s 1st day of last menstrual period and/or 1st trimester ultrasonography. Fetal growth restriction of the baby was defined as birth weight below the 10th percentile for the gestational age. Venous blood samples were taken and transported to the biochemistry laboratory. Serum separation was done without delay and stored at appropriate temperature. Length of Infant was measured with an Infantometer. Non-stretchable plastic tape was used to measure head circumference of Infants.

Results: Total 102 pregnant mothers in their third trimester were enrolled in this study for final analysis. Maternal serum 25(OH) D<12 ng/ml was found in 19% of the mothers and 46% mothers had values 12-20 ng/ml. This means 65% of women in their 3rd trimester were not having sufficient Vitamin D3 in their body. Maternal Mean serum PTH was 27.1 (SD: 21.23). However, maternal serum PTH showed a significant negative correlation with maternal 25(OH) D (r= -0.317, p= 0.0007). Further, median level of PTH was significantly higher (p=0.001) in 25 (OH) D deficient group (21.9 pg/ml) when compared to non-deficient group (11.17pg/ml) according to Mann-Whitney test. PTH showed a significant correlation to calcium, alkaline phosphatase and inorganic phosphorus according to Spearman’s correlation coefficient test. Anthropometry of the infants at birth and at one month of age was taken. Correlation between biochemical parameters (vitamin D, PTH) and growth (weight, length, OFC) was not significant.

Conclusion: A significant rate of vitamin D deficiency was observed in pregnant mothers. There was no correlation between maternal vitamin-D3 levels in 3rd trimester of pregnancy and neonatal anthropometry in this study.

Keywords: vitamin D Deficiency, PTH(parathormone), Anthropometry, pregnancy, offspring

Introduction

Pregnancy induces a natural physiological challenge to the mother, with many systems and functions of the human body adapting to this unique temporary environment. One such...
system is the regulation of vitamin D and its metabolites. PTH stimulates osteoclastic bone resorption and distal tubular calcium reabsorption and mediates 1,25-dihydroxyvitamin D (1,25(OH)₂ D) intestinal calcium absorption. Vitamin D stimulates intestinal absorption of calcium, regulates PTH release by the chief cells, and mediates PTH-stimulated bone resorption. Vitamin D, especially its most active metabolite 1,25 dihydroxyvitamin D₃, plays an important role not only in calcium homeostasis and bone remodelling, but also in the control of hormone secretion, immune dysfunction, cell-proliferation and differentiation.¹

Regulation of the vitamin D system is also important for extra-skeletal functions including the enhancement of muscle cell contractility, immunity, cognitive capacity and cardiometabolic health.²⁻⁶ Immunomodulatory and anti-inflammatory properties of vitamin D have been demonstrated, particularly in conditions of chronic low-grade inflammation such as type 2 diabetes⁷ and heart failure⁸, as well as in autoimmune diseases including asthma.⁹ Vitamin D requirements are increased during pregnancy to adapt to heightened physiological demands in the mother, including driving the formation of the fetal skeleton and maintaining an environment of tolerance to paternal and fetal tissue and their accompanying alloantigens.¹⁰,¹¹ Deficiency in vitamin D during the physiologically and metabolically challenging period of pregnancy has been associated with several adverse pregnancy and childhood outcomes including gestational diabetes, pre-eclampsia, preterm birth, childhood asthma and impaired psychomotor and cognitive development.¹²⁻¹⁴ However, many of these associations have been inconsistently reported in the literature, despite extensive research into vitamin D in the context of pregnancy. Furthermore, the lack of clarity regarding optimal vitamin D levels in relation to pregnancy outcomes has led to inconsistencies in the guidelines for classifying vitamin D deficiency or defining the level of supplementation required to support a healthy pregnancy.¹⁵ Most of the previous literature examining vitamin D in pregnancy has been limited to a single measure of vitamin D, that is, total 25-hydroxyvitamin D (25(OH)D). However, attempts to understand the complexities of the vitamin D metabolic system and its effects in pregnancy have led to the exploration of other metabolites within the vitamin D system. More novel components of the vitamin D system, such as the vitamin D-binding protein (VDBP), the primary carrier protein of vitamin D, have recently been identified as potential targets for research. This is due to an increased understanding of the potential role of VDBP in the physiology underlying vitamin D deficiency and the metabolic changes in pregnancy.¹⁶ VDBP concentrations have been shown to increase dramatically in pregnancy and thus influence the biologically and functionally active portion of vitamin D, free vitamin D, which is postulated to be more representative of vitamin D status in the pregnancy state than total 25(OH)D. VDBP itself has been implicated as a potential biomarker of pregnancy outcomes as it has been linked to several adverse outcomes including gestational diabetes, pre-eclampsia and preterm labour, although the present literature exploring this protein remains very limited.¹⁷ Importantly, VDBP has been linked to various biological processes that are often exacerbated or heightened in the pregnant state, including immunoregulation, glucose metabolism, and regulation of blood pressure.¹⁸ VDBP is also involved in maintaining an environment of tolerance to paternal and fetal tissue and the augmentation of pro-inflammatory states.¹⁶ These largely unexplored components of the vitamin D metabolic system provide alternative and novel avenues for improving our understanding of the functions of vitamin D in pregnancy, and potentially optimising pregnancy outcomes.

Material and Methods

A prospective observational study was conducted in the Department of Obstetrics & Gynaecology and Department of Pediatrics, Narayan Medical College and Hospital,
Sasaram, Rohtas, Bihar, India, between February 2019 to January 2020, after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent, detailed history was taken from the patient or the relatives if the patient was unable to answer. The technique, risks, benefits, results and associated complications of the procedure were discussed with all patients.

**Methodology**

Convenient sampling technique was employed and all mothers attending the antenatal clinic in their 3rd trimester, after taking the proper informed consent were enrolled in this study. Exclusion criteria were mothers already on vitamin D supplements, multiple pregnancy, serious medical problems (non-obstetric) and disability that could be related to bone metabolism. We collected data on demography, obstetric history, general health and past medical and surgical conditions, details of diet, sun exposure, medications and nutritional supplements. Gestational age was calculated using mother’s 1st day of last menstrual period and/or 1st trimester ultrasonography. Fetal growth restriction of the baby was defined as birth weight below the 10th percentile for the gestational age. Eligible mothers were interviewed and a brief clinical examination performed. Their weights and heights were recorded. Venous blood samples were taken and transported to the biochemistry laboratory. Serum separation was done without delay and stored at appropriate temperature. Follow up included taking anthropometric measurements of the baby at birth and at one month of age. Length of babies was measured with an Infantometer. Non-stretchable plastic tape was used to measure head circumference of babies. We also collected information on baby’s feeding, general health and supplementation received. Analysis of 25-(OH) D was done by VIDAS® 25 OH Vitamin D Total, in serum using the Enzyme Linked Fluorescent Assay (ELFA). It is very well correlated to the Liquid Chromatography-Mass Spectrometry/Mass Spectrometry reference method with cross reactivity of 100% with 25 OH Vitamin D3 and 91% with Vitamin D2. Analysis of calcium, phosphate and alkaline phosphatase was done using the colorimetric method. The DRG (EIA-3645) Intact-PTH ELISA was employed for quantitative determination of intact-PTH in serum.

SPSS version-20 was used for statistical analysis. We applied Spearman correlation to study the influence of 25(OH) D and parathyroid hormone (PTH) on weight, length, and head circumference (OFC) at birth and at one month of age. Relationship between neonatal anthropometric measures and maternal biochemical parameters was analysed using Spearman correlation and non-parametric tests.

**Results**

Total 102 pregnant mothers in their third trimester were enrolled in this study for final analysis. Cut off values for 25(OH) D deficiency was taken as <12 ng/ml, insufficiency 12-20ng/ml and sufficiency >20ng/ml, according to Institute Of Medicine (IOM) report and the consensus report on nutritional rickets. Maternal serum 25(OH) D<12 ng/ml was found in 19% of the mothers and 46% mothers had values 12-20 ng/ml. This means 65% of women in their 3rd trimester were not having sufficient Vitamin D₃ in their body (Fig.1). 25(OH)D levels, parathyroid hormone (PTH) levels and bone biochemistry of the respondents are shown in Table1.
Table 1: 25 (OH) D levels, parathyroid hormone levels and bone biochemistry of the respondents

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 (OH) D (ng/ml)</td>
<td>19.2 (7.9)</td>
</tr>
<tr>
<td>Serum total calcium (mmol/L)</td>
<td>2.5 (0.3)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>185.7 (54.11)</td>
</tr>
<tr>
<td>Parathyroid hormone (pg/ml)</td>
<td>27.1 (21.23)</td>
</tr>
<tr>
<td>Inorganic phosphorus (mmol/L)</td>
<td>1.30 (0.18)</td>
</tr>
</tbody>
</table>

Maternal Mean serum PTH was 27.1 (SD: 21.23). However, maternal serum PTH showed a significant negative correlation with maternal 25(OH) D ($r = -0.317, p= 0.0007$). Further, median level of PTH was significantly higher ($p=0.001$) in 25 (OH) D deficient group (21.9 pg/ml) when compared to non-deficient group (11.17pg/ml) according to Mann-Whitney test. Relationship of 25 (OH) D and PTH to other biochemical parameters are shown in Table 2.

**Table 2: Correlation of parathyroid hormone (PTH) and 25 (OH)D with other biochemical parameters**

<table>
<thead>
<tr>
<th>PTH</th>
<th>Calcium</th>
<th>Alkaline phosphatase</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>r-value</td>
<td>-0.261</td>
<td>0.219</td>
<td>-0.419</td>
</tr>
<tr>
<td>p-value</td>
<td>0.021</td>
<td>0.041</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r-value</td>
<td>0.116</td>
<td>-0.072</td>
<td>0.242</td>
</tr>
<tr>
<td>p-value</td>
<td>0.315</td>
<td>0.527</td>
<td>0.028</td>
</tr>
</tbody>
</table>

PTH showed a significant correlation to calcium, alkaline phosphatase and inorganic phosphorus according to Spearman’s correlation coefficient test. Anthropometries of the infants at birth and at one month of age are given in Table 3.

**Table 3: Anthropometry of infants at birth and at one month of age**

<table>
<thead>
<tr>
<th>Anthropometry</th>
<th>At birth: Mean (SD)</th>
<th>At one month: Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>3.11 (0.7)</td>
<td>4.2 (0.7)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>51.5 (6.6)</td>
<td>55.7 (4.4)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>32.5(4.2)</td>
<td>36.9(1.5)</td>
</tr>
</tbody>
</table>

Correlation between biochemical parameters (vitamin D, PTH) and growth (weight, length, OFC) was not significant. No confounding effect of gestational age, sex of the infant, maternal height on infant anthropometry was found with 4-way ANOVA. We grouped maternal vitamin D levels into insufficient/deficient (<20ng/L) and sufficient categories. There was no significant effect on growth parameters of the infant even if the mother showed vitamin D insufficiency/deficiency (Table 4).

**Table 4: Comparison of birth parameters of infants with maternal 25(OH)D sufficiency and insufficiency/deficiency**

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>Length (cm)</th>
<th>OFC (cm)</th>
<th>Maternal height</th>
<th>Gestational age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient/deficient (&lt;20ng/ml)</td>
<td>3.0877</td>
<td>51.76</td>
<td>32.61</td>
<td>154.5</td>
<td>38.2</td>
</tr>
<tr>
<td>Sufficient (&gt;20ng/ml)</td>
<td>2.8784</td>
<td>51.54</td>
<td>33.12</td>
<td>153.2</td>
<td>38.1</td>
</tr>
<tr>
<td>p value</td>
<td>0.054</td>
<td>0.87</td>
<td>0.45</td>
<td>0.262</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Discussion

Low maternal vitamin D levels during pregnancy have been associated with a number of adverse neonatal outcomes, including small for gestational age (SGA), preterm birth, detrimental effect on offspring bone and teeth development, and risk of infectious diseases. A growing body of observational studies indicated that maternal hypovitaminosis D (as defined by maternal 25-hydroxyvitamin D [25(OH)D] levels < 20 ng/ml) is a significant risk factor of adverse neonatal outcomes.\textsuperscript{20}

In the present study we examined the relationship of PTH to serum 25(OH) D. There was a significant negative correlation between PTH and 25(OH) D. Yet, very few had PTH values above 65pg/ml and expected rise in PTH was not seen with maternal vitamin D insufficiency. More information on vitamin D receptor function and genetic composition influencing bone metabolism is needed. Further, re-evaluating vitamin D cut off levels for our population is worthwhile.

Adverse effects of vitamin D are many. However, in contrast to evidence from some studies, we could not demonstrate any effect of maternal 25-(OH) D in the third trimester on anthropometry of the infant.\textsuperscript{23,24} According to Morley et al low maternal 25-(OH) D in late pregnancy is associated with reduced intrauterine long bone growth.\textsuperscript{24} Same author has suggested in another report that the relationship between vitamin D level and birth size may require considering vitamin D receptor genotype when interpreting.\textsuperscript{25} Thus, it is suggested that not only the vitamin D level but other factors like vitamin D binding protein and vitamin D receptor may be influencing the association between growth of fetus and 25(OH)D. It indicates that vitamin D supplementation needs to be titrated carefully, since hypervitaminosis D can suppress the bone growth. Findings of few other studies resembled our results; being unable to show a relationship.\textsuperscript{26-28} It is clear that there is lack of consensus and this conflicting evidence could be due to variations of study designs, timing of vitamin D testing during pregnancy, cut-off point of vitamin D deficiency, ethnicity and genetic variation of the study population.
In this study the mean serum PTH was 27.1 (SD: 21.23). Maternal serum PTH showed a significant negative correlation with maternal 25(OH) D ($r = -0.317, p = 0.0007$). Further, median level of PTH was significantly higher ($p=0.001$) in 25 (OH) D deficient group (21.9 pg/ml) when compared to non-deficient group (11.17pg/ml) according to Mann-Whitney test.

High PTH levels are usually considered as a sign of stress to calcium metabolism. The result of this stress could lead to low fetal growth. Scholl et al has reported that maternal calcium metabolic stress, rather than low calcium or insufficient vitamin D, adversely affects fetal growth. However, present study could not report a relationship between high PTH affecting neonatal anthropometry.

There were several limitations in this study. Since we measured 25(OH) D levels only in the 3rd trimester, which does not correspond to vitamin D status in early pregnancy. Since this was a preliminary study and was conducted in a teaching hospital in one district, it is not suitable to generalise these findings to other regions in the country.

Conclusion
A significant percentage of pregnant women in this study had vitamin D deficiency/insufficiency. There was no correlation between maternal vitamin-D3 levels in 3rd trimester of pregnancy and neonatal anthropometry in this study. Since PTH rise was not significant in 25 (OH) D deficient mothers, whether the cut-off value for 25(OH) D should be adjusted to suit our population of pregnant mothers remains to be elucidated in future studies. We concluded that neither 25(OH) D nor PTH levels associated with growth parameters of the offspring. However, possible involvement of other mechanisms at receptor level or vitamin D binding proteins warrants further investigation.

Reference
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