Original article:

Correlation of vitamin D with different trimesters of pregnancy

Surabhi Puri*, Dr. Ipsita Chaudhary, Dr. Pallavi Anand, Dr. U.N Singh

Department of Biochemistry and OBGY, RAMA MEDICAL UNIVERSITY Hospital, Kanpur.
Corresponding author *

Abstract

Introduction: Maternal vitamin D status is important during pregnancy, as mother is the sole source of vitamin D substrate for her developing fetus. Maternal Vitamin D deficiency is associated with subtle fetal bone abnormalities, low birth weight and high risk of small gestational age.

Material and Method: Upon ethical clearance, a randomized control study was conducted on 100 patients of 1st, 2nd and 3rd trimesters of pregnancy attending the Antenatal clinic of the department of Obs. and Gynaecology of RAMA MEDICAL UNIVERSITY Hospital, Kanpur. 5ml of centrifuged serum was used in COBAS E 411 under appropriate temperature for the detection of vitamin D by Chemiluminescence method.

Result: A highly significant difference was obtained between II and III trimesters as well as I and III trimester.

The levels of vitamin D were found to be highly decreased in 3rd trimester as compared to 1&2nd trimester of pregnancy. This can be explained by the fact that initially in the I trimester the vitamin D levels has to be synthesized more for the requirement in pregnancy. And as the pregnancy advances vitamin D level decreases, for the need of fetal growth.

Conclusion: A statistically significant association was obtained between the Vitamin D levels of II vs III as well as III vs I trimester.

Keywords: Vitamin D, pregnancy, vitamin D deficiency, cholecalciferol

Introduction

Vitamin D refers to a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. In humans, the most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol), cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. Angus and coworkers isolated vitamin D in 1931 and named it as Calciferol, which was later identified as vitamin D3. The structure elucidation was done independently by Otto Diels and Kurt Alder. Both were awarded Nobel prize in 1950. The duration of pregnancy has traditionally been calculated by the clinicians in terms of 10 lunar months or 9 calendar months and 7 days or 280 days or 40 weeks, calculated from the first day of the last menstrual period. This is called menstrual or gestational age.

Fertilization occurs 14 days prior to the expected missed period and in a previously normal cycle of 28 days duration, it is about 14 days after the first day of the period. Thus, the true gestation period is to be calculated by subtracting 14 days from 280 days, i.e. 266 days. This is called fertilization or ovulatory age.

Pregnancy is divided into 3 trimester[4] which include;

First Trimester (week 1 to 12)
During the first trimester, the placenta develops. It is a round, flat organ that transfers nutrients from mother to the baby and transfers baby’s wastes. A primitive face takes form with large dark circles for eyes. The mouth, lower jaw, and throat are developing. Blood cells are taking shape, and circulation will begin. By the end of the first month, mother’s baby is about 1/4 inch long - smaller than a grain of rice.

**Second Trimester (13 to 28 week)**

At 16 weeks, and sometimes at 12 weeks muscle tissue, bone and skin of the infant starts forming. At 20 weeks a woman can feel movement. At 24 weeks footprint and fingerprints starts forming and the fetus sleeps and wakes regularly.

**Third Trimester (29 to 40th week)**

At 32 weeks, the bones becomes soft and almost fully formed, late preterm births occur between 34 and 36 weeks\(^5\). Infants born at 39 or 40 weeks of pregnancy are considered full term. These infants have better health and outcomes than do infants born earlier or later than this period.

![Figure 1: Different trimesters of pregnancy](image)

Vitamin D is a secosteroid and the most significant source is direct exposure of the skin to sunlight. It can also be obtained from dietary sources, nutritional supplementation and food fortification. Very few foods naturally contain vitamin D and foods that are fortified with vitamin D are often inadequate to satisfy either a child's or an adult's vitamin D requirement. The major source of vitamin D for both children and adults is from sensible sun exposure. Skin color and modern practices of cosmetic dermatology (sun tan lotions and creams, etc.) have significantly rendered the sun redundant. In the skin, photolysis by UV-B-photons(290-315nm) between position 9 and 10 of the steroid ring leads to opening of the ring B to give the provitamin, secosterol. The cis double bond between 5th and 6th carbon atoms, is then isomerised to a trans double bond to give rise to vitamin D\(_3\) or cholecalciferol. So vitamin D is called as “**sun shine vitamin**”. In the liver, cholecalciferol (vitamin D\(_3\)) is converted to calcifediol. **Ergocalciferol** (vitamin D\(_2\)) is converted in the liver to 25-hydroxyergocalciferol. These two specific vitamin D
metabolites are measured in serum to determine a person's vitamin D status. Part of the calcifediol is converted by the kidneys to calcitriol, the biologically active form of vitamin D. Calcitriol circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. Calcitriol also affects neuromuscular and immune function.

**Vitamin D levels in Pregnancy**

Mother is the sole source of vitamin D substrate for her developing fetus, during pregnancy than any time of the life cycle. Intestinal calcium absorption, are only increased during the 3rd trimester. Fetal calcium levels are higher than maternal throughout gestation. During pregnancy, vitamin D values tend to decrease from their optimal requirements for the body for several reasons. This phenomenon may lead to developing either vitamin D insufficiency or deficiency, which may affect the mother directly and later the offspring. There are specific risk factors that have the main influence to predispose vitamin D deficiency (VDD) among pregnant women. The fetal skeleton begins to calcify in the last trimester, thereby increasing maternal demand for calcium. This demand is met by increased production of 1, 25(OH)₂ D by the mother's kidneys and placenta. Circulating concentrations of 1, 25(OH)₂ D gradually increase during the 1st and 2nd trimesters, owing to an increase in Vitamin D-binding protein concentrations in the maternal circulation. However, the free levels of 1, 25(OH)₂ D, which are responsible for enhancing transport of calcium across the placenta. Maternal Vitamin D deficiency is associated with subtle fetal bone abnormalities like shorter knee-heel length, low birth weight and high risk of being small for gestational age.

- Sufficiency: 25(OH)D > 30 ng/ml
- Insufficiency: 25(OH)D 20-30 ng/ml
- Deficiency: 25(OH)D < 20 ng/ml

**Role of vitamin D in Pregnancy**

Low levels of vitamin D in pregnancy are associated with gestational diabetes, pre-eclampsia, and small infants. The benefit of supplements, however, is unclear. Vitamin D deficiency is a common condition among high risk pregnant women and their children. The routine monitoring of serum 25(OH)D₃ levels in antenatal period is mandatory. Pregnant women who take an adequate amount of vitamin D during gestation may experience positive immune effects.

**Why do we need Vitamin D during pregnancy?**

Vitamin D is beneficial for our own personal health. It has now extensive research supporting its role in immune function, healthy cell division and bone health. It is necessary for the absorption and metabolism of calcium and phosphorus. Many studies are finding a connection between low serum vitamin D levels and an increased risk of certain types of cancers, autoimmune disease, neurological disease, insulin resistance, and cardiovascular disease.

**Correlation of vitamin D with pregnancy**

The conversion of vitamin D to 25(OH)D appears unchanged during pregnancy, following first- and zero-order enzyme kinetics; by contrast, the conversion of 25(OH)D to 1,25(OH)₂D during pregnancy is unique and unparalleled during life. At no other time during life is 25(OH)D so closely linked with 1,25(OH)₂D. By 12 weeks of gestation, 1,25(OH)₂D levels are more than twice that of a nonpregnant adult and continue to rise two- to
threefold from the non pregnant baseline rising to over 700 pmol/l, attaining levels that would be toxic due to hypercalcemia to the non pregnant individual, but which are essential during pregnancy\textsuperscript{[16,17,18]}. Adequate nutritional vitamin D status during pregnancy is important for fetal skeletal development, to the enamel formation, and perhaps general fetal growth and development. There also is mounting evidence to suggest that vitamin D deficiency impacts on the fetal immune function, not only of the mother.

**Material and methods**

**Study design:**- Experimental study design with comparative study method.

Upon ethical clearance, the study was a randomized control study on 100 patients of I, II And III trimesters of pregnancy attending the Antenatal clinic of the department of Obstetrics and Gynecology of RAMA MEDICAL COLLEGE hospital, Kanpur. After informed consent patients were asked for the blood sample which were taken in collection room under the ethical conditions. 5 ml of serum was collected under aseptic conditions. Further centrifuged serum was used in COBAS E 411 under appropriate temperature for the detection of vitamin D by Chemiluminescence method.

**Statistical Analysis**

Statistical Analysis was done by SPSS software (Statistical Package for the Social Science)\textsuperscript{2.1}

**Results**

Distribution of trimesters according to the samples;

<table>
<thead>
<tr>
<th>Trimester</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>30</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

**Table I:** trimesters of pregnancy.

![Pie chart showing distribution of trimesters](chart.png)
Figure 2: Pie chart showing distribution of trimesters

<table>
<thead>
<tr>
<th></th>
<th>I trimester</th>
<th>II trimester</th>
<th>III trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>10.96</td>
<td>12.46</td>
<td>7.73475</td>
</tr>
<tr>
<td>STDev</td>
<td>6.51140</td>
<td>7.34321</td>
<td>3.7396</td>
</tr>
</tbody>
</table>

Table II: Mean and STDev

Vitamin D levels in I trimester

Graph 1: Vitamin D level in I trimester

Vitamin D levels in II trimester

Graph 2: Vitamin D level in II trimester
Vitamin D levels in III trimester

Graph: Vitamin D level in III trimester

Correlation of vitamin D with different trimesters

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Correlate</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>II Trimester</td>
<td>-1.49733</td>
</tr>
<tr>
<td></td>
<td>III Trimester</td>
<td>3.22925</td>
</tr>
<tr>
<td>II</td>
<td>I Trimester</td>
<td>1.49733</td>
</tr>
<tr>
<td></td>
<td>III Trimester</td>
<td>4.72658</td>
</tr>
<tr>
<td>III</td>
<td>I Trimester</td>
<td>-3.22925</td>
</tr>
<tr>
<td></td>
<td>II Trimester</td>
<td>-4.72658</td>
</tr>
</tbody>
</table>

Table III: Correlation of vitamin D with different trimesters

p value of different trimesters

<table>
<thead>
<tr>
<th>Trimester</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vs II</td>
<td>0.596</td>
</tr>
<tr>
<td>I</td>
<td>0.069</td>
</tr>
<tr>
<td>II</td>
<td>0.596</td>
</tr>
<tr>
<td>II Vs I</td>
<td>0.004</td>
</tr>
<tr>
<td>III</td>
<td>0.004</td>
</tr>
<tr>
<td>III Vs I</td>
<td>0.069</td>
</tr>
<tr>
<td>III</td>
<td></td>
</tr>
</tbody>
</table>

Table IV: p value of different trimesters
Discussion

The conversion of vitamin D to 25(OH)D appears unchanged during pregnancy following first and zero order enzyme kinetics\cite{14,15}. 25(OH)D circulates bound to the vitamin-D-binding protein, has a 2-week half-life, and is an indicator of the endogenous vitamin D status. It may be further hydroxylated to bioactive 1,25-dihydroxyvitamin D [1,25(OH)2D]. During pregnancy, the decidual, placental, and maternal renal synthesis of 1,25(OH)2D increases the maternal serum levels of 1,25(OH)2D\cite{19,20}, thereby enhancing the maternal calcium absorption in order to fulfill fetal needs\cite{21}. The human fetus seems to be protected against excessive maternal 1,25(OH)2D increases\cite{22}.

Adequate nutritional vitamin D status during pregnancy is important for fetal skeletal development to the enamel formation and perhaps general fetal growth and development. Among 100 subjects; pregnant ladies were divided into 3 trimesters , which included 30 in I trimester,30 in II trimester and 40 in III trimester( Table I). And mean standard deviation was taken (table II) Our study was to correlate 1\textsuperscript{st},2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy (figure 1). Total 100 patients participated in the study(figure no.2), who were agreed to take vitamin D supplement if deficient . In a study by Merewood et.al\cite{23}, the risk of pre-eclampsia was 5 fold increased among the women with 25(OH)D levels below 15ng/ml at a pregnancy duration of upto 22 weeks.

According to the recent studies, population is found to be more vitamin D deficient who are of darker pigment, who had migrated from sunny climates or who wore clotting that left little skin exposed or who used suns cream. As vitamin D is a unique nutrient because its requirement can be met by both exogenous production from sunlight as well as exogenous dietary source, which complicates determining the body’s daily nutrients requirements. Methods are currently available to quantify the contribution of endogenous vitamin D synthesis resulting from sun exposing but serious limitations remain in accurately estimating dietary vitamin D intake because of the incompleteness of nutrients data base for both vitamin D fortified food and vitamin D supplements.

Correlation of vitamin D with different trimesters

Significant difference between II and III trimester can be explained by the fact that vitamin D levels are continuously being utilized by the fetus leading to decrease in the level of vitamin D in the III trimester. Our study correlated with . Datta et al who showed that vitamin D-deficient pregnant women who consumed less than 1,600 IU/day of vitamin D could not maintain sufficien Significant difference was also found between 1 and III trimester. This can be explained by the fact that initially in the I trimester the vitamin D levels has to be synthesized more for the requirement in pregnancy. But as compared to the III trimester , the levels of I trimester were still found to be less. In a study by Perez et al.,\cite{24} it was observed that only 35.9% of pregnant women in the first trimester had adequate serum 25(OH)D levels (≥30 ng/mL) and non-Caucasian ethnicity, season at sampling (autumn/winter) and null parity were found to be cy at the end of pregnancy\cite{25} associated with 25(OH)D deficiency. Bodnar et al.\cite{26} reported a five-fold increased risk of pre-eclampsia in women with 25(OH)D <15 ng/mL at <22 weeks of pregnancy.

The p value between I and II trimester was found to be insignificant. This can be explained by the fact that there was less difference in the vitamin D levels between I and II trimester. At the same time during II trimester the fetus starts utilizing maternal vitamin D.
According to our study we concluded that pregnant women are vitamin D deficient from 1st trimester due to lack of sunlight or vitamin D nutrient. Many of them are not aware of vitamin D deficient due to which they result in incomplete pregnancy or pre-eclampsia because in the 2nd trimester of pregnancy; fetus needs mother’s vitamin D for its growth and development, but if the mother is deficient from the 1st week of pregnancy how vitamin D will be synthesized in her body for the fetus. In this study we found among I, II and III trimester, in III trimester mother’s are more deficient than I ,II trimester. As we can say vitamin D is used up by the fetus for its requirement in the 2nd trimester. As a result as trimester of pregnancy advances; women become more vitamin D deficient.

Pregnant women should get vitamin D tested as soon as pregnancy starts and should be prescribed vitamin D supplement if deficient with proper exposure of sunrays. To avoid incomplete pregnancy or fetal retardation.

CONCLUSION

1. Vitamin D levels were analyzed in different trimesters of pregnancy.
2. A statistically significant association was obtained between the Vitamin D levels of II vs III trimester as well as III vs I trimester.
3. No significant difference was found in the vitamin D levels between I and II trimester.

Acknowledgment

Doing research was a cumbersome job, but acknowledging all those who helped in this apparently impossible task is a pleasure to us.

Words are not enough to express my gratitude for our family, friends. They were always there cheering us and stood by us through the good times and bad. We would never have been able to finish our work without the guidance of our committee members, technicians and support from relatives.

We can never forget to thank our patients without whom our work could have never started.

Last but not the least we are thankful to Almighty God, who is a silent inspiration to all good work and without whose benevolence and blessings nothing can ever be achieved.

Bibliography

2. Calvo MS, Whiting SJ, Barton CN; Whiting; Barton (February 2005). "Vitamin D intake..j Nutr.2005:310-6
23. Merewood association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab. 2009