Early pregnancy vitamin D deficiency and risk of preterm Birth.

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Abstract

**Background:** Many researches done for publication on mineral and vitamins during pregnancy and the possible influences of supplements on pregnancy outcome. Vitamin D may play roles in pregnancy and its complication.

**Aim:** To estimate the association between maternal 25-hydroxy vitamin D concentration in early pregnancy and risk of preterm birth.

**Sitting:** In Al-Zahraa Teaching Hospital in Al-Najaf City from March 1st 2015-September 30th 2015.

**Type of study:** Prospective cohort study

**Method:** 300 pregnant women were included in the study. Blood sample from each case withdrawn from pregnant women 14- 20 weeks of gestation, sent for vitamin D assay. Cases were followed during pregnancy to identify patients who will develop preterm labour.

**Results:** The result of this study showed that 300 pregnant women was with deficient or had insufficient vitamin D level. The relative risk is 6 times which mean that those who had deficient or insufficient vitamin D level 6 times more likely to end with preterm delivery.

**Conclusion:** In this study found that there is strong association between.

**Keywords:** pregnancy, vitamin D, deficiency, preterm birth


Introduction

I. Preterm pregnancy

Preterm birth refers to a birth that occurs before 37 completed weeks (< 259 days) of gestation. A very preterm birth is more variably defined as less than 32 - 34 weeks of gestation, and an extremely preterm birth is a birth at less than 28 weeks of gestation (1). The **incidence** of preterm birth in the developed world is range between 7-12%. The rate of preterm birth prior to 32 weeks has remained relatively stable at 1-2 % (2). Many factors contributed to the increase in preterm birth (3,4,5). In most cases, the cause of preterm labor is not diagnosed, and this is likely to be multifacatorial (6,7,8). **Prediction:** (a)clinical risk
scoring: The patient's obstetric history may provide clue as to whether women at increase risk of premature delivery, as young women less than 16 years of age, uterine abnormalities, women of low socioeconomic class, who have had a previous preterm birth the risk in the next pregnancy will approximately 20%, uterine over distension as polyhydromnious and multiple pregnancy (approximately 50% of twins and 90% of triples are born preterm), premature rupture of membranes, cigarette smokers, abusers as alcohol or cocaine, women with low body mass index and women with bacterial vaginosis (3,7,10). Several clinical risk scoring systems to predict preterm labour have been devised based on these epidemiological observations (11). Although various risk scoring systems have been suggested, they have low positive predictive values, and are therefore of limited value in identifying women with a significant risk of preterm labour (12). (b)- Cervical length before the onset of labour, the cervix shortens and softens (13). ©-Fetal fibronectin is an extracellular matrix glycoprotein produced by the chorionic cells. It is normally present in the vaginal secretions until 20 weeks, and then disappears from the cervicovaginal secretions, only to appear before the onset of labour at term. If the adhesive fibronectin interface between the chorion and the decidua is damaged, fetal fibronectin my reappear in the vaginal secretions earlier, and it's detection has therefore been proposed as a predictor of preterm labour. It's value lies in it's high negative predictive value and prevent over treatment (negative predictive value for delivery within 14 days in 97%) (14). (d) Salivary Oestriol may be helpful (15). (e-) Home uterine activity monitoring; showed a reduced risk of preterm labour with home uterine activity monitoring device (16).

Clinical presentation: The three general clinical presentations of preterm labour are: Spontaneous preterm labour with intact membranes, preterm pre labour rupture of membranes and iatrogenic. A vague complaint such as increased discharge, pelvic pressure or low backache are sometime reported (17).

Perinatal mortality and morbidity: The preterm birth accounts for over 85% of perinatal morbidity and mortality. The morbidity, mortality and costs of preterm delivery are higher at lower gestational ages (3,10,18). Regarding maternal mortality and morbidity; as feeling inadequacy at fulfilling a reproductive role and the development postpartum endometritis (19).

II- Vitamin D

It is a steroid vitamin from a group of fat-soluble vitamin. It is naturally present in very few foods, so that it is available as a dietary supplement. It is also produced endogenously when ultra-violet rays from sun light strike the skin and trigger vitamin D synthesis (20).

It is known that vitamin D. and pregnancy are important together, expecting that the mothers need to make sure they get the recommended amounts of vitamin D during pregnancy for both their own wellbeing and healthy development of their baby (21).

Vitamin D is beneficial for personal health. It has role in immune function, healthy cell division and bone health. They found low serum vitamin D levels association with an increased risk of certain types of cancers, autoimmune disease, neurological disease, insulin resistance, and cardiovascular diseases. It is necessary for the absorption and metabolism of calcium and phosphorus, in addition the deficiency with vitamin D is also related to preeclampsia (23).
At this time, 40-60% of the entire U.S population is vitamin D deficient including pregnant women. There is a very short list of food that contain vitamin D (24), as egg yolk, salmon and cod liver oil, however, most vitamin D is consumed through fortified foods like milk. For 75% of the population have lactose intolerant, fortified milk products are not a reliable source of vitamin D consumption (25).

Additionally many factors influence the body's ability to make and absorb vitamin D. include: where you live, the season, how much time spend out doors without sun screen research suggests sun exposure (usually 5-10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3times per week) can help as well , skin pigmentation, age, obesity, and pollution(26). However, the best way to really ensure adequate vitamin D is through simple supplementation, which are two forms D2 and D3 (21).

Ergocalciferol is the vegetarian from the vitamin D and cholecalciferol is the animal-sourced usually derived from fish liver oil or lanolin from sheep. Cholecalciferol from the most absorbable and utilized form for the body(27).

Vitamin D deficiency is a common problem in reproductive age women in industrialized countries (28), as those who live in cold climate, northern, latitudes, or wear sun and winter protective clothing, and ethnic minorities especially those with darker skin(29).

Vitamin D deficiency is also more common among heavier women than leaner individuals(30).

During pregnancy severe maternal vitamin D deficiency as defined as serum 25 - hydroxy vitamin (25-OH.vitamin D) concentration less than 5ng/ml has been associated with disordered skeletal homeostasis as congenital rickets and fractures of the newborn(31) however, the effect of less severe vitamin D deficiency and insufficiency on maternal and fetal outcomes during pregnancy is less clear, recent observational and randomized control trials have attempted to investigate this issue, but there remains limited guidance on the management of vitamin D deficiency during pregnancy. During pregnancy severe maternal vitamin D deficiency as defined as serum 25- hydroxyvitamin (25-OH.vitamin D) concentration less than 5ng/ml has been associated with disordered skeletal homeostasis as congenital rickets and fractures of the newborn(31).

Aim of study to estimate the association between maternal 25-hydroxy vitamin D concentration and risk of pre term birth.

Patients and methods: A prospective cohort study is done in Al-Najaf City, Al-Zahraa Teaching Hospital attached to Medical College/Kufa University from 1st March - 30th September 2015. The study done on pregnant women with gestational age between 14-20 weeks gestation who attend the out patient clinic and emergency room involved in our study. Total number of collected cases this study was 320 pregnant women. All women agreed to participate in this study by verbal and written consent. However, only 300 pregnant women continue to follow this study.

The criteria of the study group: Gestational age between 14-20 weeks with mean (17.94) , gravity range between 1-5 with mean (2.05) , their age range was between 18-32 years old with mean (25years). BMI was ranged between 20-26 kg/ m2 with mean (22.77kg/m2).
The exclusion criteria are as follow: History of diabetes, essential hypertension, renal disease or any other medical disease, smoking, and all cases had no history of taking of vitamin D supplement.

According to Medicine and Endocrine Society criteria for vitamin D level in blood categorization are: >30 ng/ml regarded as sufficient, 21-29ng/ml regarded as insufficient and < 20 ng/ml regarded as deficient.(22)

Measurement of Vitamin D: A 5ml of blood was taken by venipuncture, plasma is separated by centrifugation of blood for 20 minutes and then the sample had been sent to laboratory. By using ELISA kit; ELISA assay were performed as soon as possible after sample collection. If ELISA assay cannot performed immediately, the plasma stored at -20ºC.

The micro ELISA strip plate provided in this kit has been precoated with an antibody specific antibody. Then Horse radish peroxidase conjugated antibody specific for hydroxyl vitamin D3 (HVD3) was added to each micro ELISA strip plate well and incubated. The TMB substrate solution was added to each well, only those wells that contain hydroxyl vitamin D3 and HRP conjugated HVD3 antibody appeared blue in color and then turn yellow after the addition of the solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of HVD3. The calculation of concentration of HVD3 in sample was compared the OD of the sample to the standard curve. Statistical analysis done by using SPSS (statistical package for social sciences) version 20. In which we use chi-square test for categorical data. We calculate relative risk for this measurement. P value < 0.05 regarded as significant.

Results: The data of 300 pregnant women was collected and analyzed. Table (1) demonstrates the demographic characteristics of patients. The mean gestational age 17.9 weeks, ranged of (14-20) weeks, with standard deviation(SD ) of 1.241 and their parity was ranged (1-5) with mean 2.05 , SD 0.846. The body BMI was ranged (20-26kg/m2), with mean (22.77) and std. deviation (1.2101. Regarding vitamin D levels were between (7.6-60 ng/ml ) with mean ( 27.739 ng/ml) and SD (11.292). In addition, the time of delivery was ranged between (28-40 weeks ) gestation with mean o(36.293) , SD( 3.475).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age(weeks)</td>
<td>14</td>
<td>20</td>
<td>17.9</td>
<td>1.24159</td>
</tr>
<tr>
<td>Parity</td>
<td>1</td>
<td>5</td>
<td>2.05</td>
<td>0.84650</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>20.00</td>
<td>26.00</td>
<td>22.7743</td>
<td>1.21019</td>
</tr>
<tr>
<td>VitaminD ng/ml</td>
<td>7.60</td>
<td>60.00</td>
<td>27.7393</td>
<td>11.29203</td>
</tr>
<tr>
<td>Delivery time(weeks)</td>
<td>28</td>
<td>40</td>
<td>36.2933</td>
<td>3.47576</td>
</tr>
</tbody>
</table>

Table 2; there are two groups of pregnant women. First group those who have low level of Vit.D (deficient ≤ 20 ng/ml and insufficient between 21-29 ng/ml) include 142(47.3%) pregnant women regarded as deficient or insufficient, out of these group of cases 75(52.8%) of pregnant women developed preterm labour while 67(47.2%) pregnant women continue to term pregnancy. P- value <0.001.

Second group of pregnant women who have sufficient level of Vitamin D in their blood (≥ 30ng/ml) they include 158 (52.88%) out of total 300 cases. Moreover, among this group only 13(8.2%)
pregnant women they developed preterm labour while remaining 145(91.8%) pregnant women continue their pregnancy until term. So there is a high rate of preterm delivery among those pregnant women with low vitamin D level in comparison to those with normal level of this vitamin. Statistically there is a high significant difference between vitamin D level in pregnant women and time of delivery. P- Value <0.001, as shown in table 2 and as histogram. The relative risk is 6.419 which mean that those who pregnant women with deficient or insufficient vitamin D level are 6 times more likely to end with preterm delivery.

Table 2: Association between plasma vitamin D level and delivery time.

<table>
<thead>
<tr>
<th>NO. (%) of patients according to Vit.D3 levels</th>
<th>Delivery time (weeks)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Full term</td>
</tr>
<tr>
<td>Deficient(≤20ng/ml) or insufficient(21-9ng/ml) 142(47.3%)</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td>Sufficient (≥30ng/ml) 158(52.88%)</td>
<td>13</td>
<td>145</td>
</tr>
</tbody>
</table>

Figure 1: A histogram show association between vitamin D and delivery time.

The blue column indicates percentage of women with the deficient or insufficient vitamin D and show the 52.8% of preterm birth while in a full term group shows 47.2%. The green column indicates sufficient vitamin D, shows 91.8% has full term birth, and only 8.2% has preterm birth.

Discussion: Preterm birth is the most important problem in modern obstetrics. In 2010 more than million died worldwide making it the second leading cause of death in children under age of 5 years. Population of women who should be at relatively lower risk for pregnancy complication.

In this study, 142 out of 300 pregnant women found with deficient or insufficient vitamin D level (47.2%). Among 142 cases 75(52.8%) pregnant women ended with preterm birth. Our study is consistent with study done by Bodnar LM et al. at Feb. 2015 who did a case cohort study on selected 2327 at random among them 1126 had preterm labor. The incidence of preterm birth was 8.6% overall.
and $p$ value < 0.01 after adjustment for maternal race and ethnicity, pre pregnancy, body mass index, season, smoking and the risk of preterm birth at less than 37 weeks of gestation significantly decrease as 25-hydroxy vitamin D increase to approximately 90nmol/l (36ng/ml).

In addition, Wagner CL, et al (35) at 2014 said that analysis achieving a 25-hydroxy vitamin D serum concentration ≥ to 40ng/ ml significantly decreased the risk of preterm birth compared to ≤ to 20ng/ ml. These finding suggest the importance of raising 25-hydroxy vitamin D level substantially above 20ng/ml reaching 40ng/ml during pregnancy would reduce the risk of preterm birth.

In our study the pregnant women with deficient or insufficient vitamin D level are 6 times more likely to end with preterm delivery as shown in table( 2) while other study found women who have low blood level of vitamin D were about 1.5 time as likely to deliver early compared to those with the highest level (31). In this study, there are large number of pregnant women with low level of Vit.D with as association with high rate of preterm birth, this could be explained , that the rate of poverty is increasing therefore, improper intake of this vitamin due to high cost( an expensive) and a very short list of food that contain vitamin D.

As far as many factors influences the on body's ability to make and absorb vitamin D, such as season, how much time spend outdoors without sunscreen, and skin pigmentation; most of the blood samples from cases in this study collected at spring time where exposure to sunlight less, the time spend outdoors without sunscreen even at sun seasons for our cases are less, because the weather in our city is too hot making the skin exposure more difficult and there is high risk of hot stroke. Furthermore, around more than 95% of study cases their body already are covered a part from their faces and hands (local habits and religious causes).

Lastly most of our cases they have dark skin from ethnical point of view as far as the darken skin people are more prone to low Vit D level (29).

In contrast, this study is not agreed with Thorp JM et al (36) using 131cases with preterm delivery at less than 35 weeks of gestation and 134 term control, and measure serum 25(OH) vit D concentration by liquid chromatography –tandem mass spectrometry (LC-MS) from sample collected at base line (16-22 weeks of gestation) the median mid –gestation serum 25(OH) vit D concentration was 67 nmol \(\mu\) , only 27% had concentration of less than 50nmol\(\mu\). In conclusion that in a cohort of women with prior preterm birth , Vit. D status at mid pregnancy was not associated with recurrent preterm birth. This deference could due to the measure serum 25(OH) vit D concentration by liquid chromatography –tandem mass spectrometry (LC-MS) while in this study ELISA assay were performed

Also Shannon K. et al. (37) incontrast to our study, they had a retrospective cohort study in first trimester from 310 nulliparous women with singleton gestation without significant medical problem and vitamin D assay were performed on banked plasma specimen. 70% of studied women was vitamin D insufficiency with serum concentration less than 30ng/ml and shows that there was no association between first trimester vitamin D level and subsequent development of adverse pregnancy complication including preterm birth.

This incompatibility can be explained that the difference was in timing of study that may affects on the level of Vitamin D ( our study was at second trimester while their study at first trimester), in addition they defined vitamin D insufficiency with serum concentration less than 30ng/ml while in our
study serum concentration less than 20ng/ml regarded as low, these causes could affect the results of the studies.

Understanding the epidemiology may help in the development of health promotion programs and guide research into etiology and treatment. Several clinical risk scoring systems to predict preterm labour have been devised based on these epidemiological observations (11).

**Conclusion:** The level of vitamin D in serum of pregnant women is associated with preterm birth

**Recommendation:** Further researches are recommended on larger sample size, for longer duration of follow up and different study design that include the impact of vitamin D supplement on the preterm birth incidence. The study can be done in different season to see the impact of sun exposure on vitamin D level.

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