"Vitamin D Level and Pregnancy Outcomes among Pregnant Women in Zagazig University Hospitals; Follow-up study"

Authors

Al-Zahraa Mohammed Soliman 1, Eman Mohamed Abd el-Sattar 2, Yosra Abd elfattah Osama 3 and Mostafa Abdo Ahmed Salem 4

1 Public Health and Community Medicine, Faculty of Medicine, Zagazig University.
2 Family medicine, Faculty of Medicine, Zagazig University.
3 Clinical Pathology, Faculty of Medicine, Zagazig University.
4 Obstetrics and Gynecology, Faculty of Medicine, Zagazig University.

ABSTRACT:

Background: Adverse maternal and neonatal outcomes as preterm delivery, preeclampsia, small for gestational age (SGA) and gestational diabetes mellitus have all been accused to be associated with low levels of maternal vitamin D during conception.

Objective: to evaluate the relation between maternal and fetal outcomes and levels of maternal vitamin D during pregnancy

Patients and methods: In total, 284 third-trimester pregnant women were included in this cohort study at Zagazig University Hospitals' Obstetric and Gynecologic Clinic. They were questioned about their pregnancy and the variables related to their vitamin D levels. Vitamin D level was measured in all participants. The participants were followed till delivery with reporting all adverse maternal, fetal, and neonatal outcomes.

Results: When comparing the association between vitamin D concentration and sun exposure, residence, dressing style, dairy product consumption, occupation, and use of multivitamins women who wore clothing uncovered and who worked outside and were exposed to the sun had significantly higher vitamin D levels. Preeclampsia and hypertension incidences were higher in females with versus without vitamin D deficiency with statistically significant values [OR (CI) 5.4 (1.4-20.2) for preeclampsia and 7.3 (1.5-34.5) hypertension].

Conclusion: high prevalence of vitamin D deficiency was reported in late pregnancy, which was linked to factors as sun exposure, employment, usage of multivitamins, and dress code. Preeclampsia and hypertension are the two negative pregnancy outcomes that have been linked to low vitamin D levels. No significant relation was detected between vitamin D level and both fetal and neonatal outcomes.

Key words: Maternal morbidity, Preeclampsia, Vitamin D level
Introduction

The fat-soluble vitamin D is a lipophilic pro-hormone that has an extensive spectrum of biological and metabolic effects. In addition to influencing bone metabolism, vitamin D also affects the respiratory, endocrine, cardiovascular, and immune systems. The body synthesizes vitamin D3 primarily from sunlight exposure, with food (vitamins D3 and D2) coming in second and third.

Researchers are concentrating at the ideal levels of vitamin D. A group of experts and the Endocrine Society consider levels between 20–29 ng/mL and level of 30 ng/ml to be sufficient level. Certain reviewers suggest achieving >40 ng/mL levels for non-classical actions. Less than 20 ng/mL of vitamin D is seen as indicative of a deficiencies of vitamin D.

Serum 25-hydroxyvitamin D [25(OH) D] < 20 ng/mL is a measure of vitamin D deficiency, which is more common in females during conception and considered a public wellness concern.

Globally, approximately one million individuals experience vitamin D deficiency. Pregnant women are regarded as a high-risk category for developing vitamin D insufficiency, with prevalence rates varying from 51.3% to 100%, due to the higher biological requirement for vitamin D during pregnancy. Perinatal death and disability rates are higher in cases of VDD in pregnant women. Chinese women (100%) and Turkish pregnant women (95.6%) had the highest proportion (>80%) of insufficiency throughout pregnancy globally. An estimated 60–80% of pregnant women in Middle Eastern nations have VDD.

The placenta provides the fetal skeleton with the maternal supply of vitamin D because it is unable to synthesize it on its own. The concentrations of calcitriol within the umbilical cord have been usually less than those obtained from mother serum because calcitriol is difficult to cross the placental barrier and the fetus has low levels of parathyroid hormone.

Hypovitaminosis D was linked to a number of unfavorable prenatal outcomes, including fetal death, intrauterine growth restriction, congenital defects, and abortion. Among the maternal side effects were gestational diabetes mellitus (GDM), preeclampsia, along with an elevated Preterm risk delivery.

The causal significance of deficient vitamin D within many pregnancy disorders has been still unknown owing to a lack of local and international research; however, vitamin D therapy may minimize subnormal newborn anthropometric measurements, GDM, preeclampsia, and premature labor. For American women between the ages of 19 and 50, including those who are pregnant, 600 IU is the recommended daily allowance (RDA) per day.

The research question under the study was “What is the state of vitamin D among pregnant female and does this state related to maternal, fetal or neonatal outcomes?”
Our hypothesis is that vitamin D deficiency is prevalent among pregnant female and may be related to adverse maternal, fetal and neonatal outcomes.

So, the current study was done to detect the relations between vitamin D levels and the maternal alongside fetal outcomes.

Patients and methods

**Study design and setting:** The current study is a cohort study.

**Study setting:** The study was conducted at Zagazig University Hospitals’ Obstetric and Gynecologic Clinic.

**Population:** After providing written consent, a total of 284 third-trimester pregnant patients with gestational ages between 37 and 40 weeks who were preparing for delivery at Zagazig University Hospitals' Obstetric and Gynecologic Clinic were included in this cohort study.

**Inclusion criteria:** Women in pregnancy between the ages of 18 and 35 who had a normal body mass index (BMI) and had not previously taken vitamin D supplements were involved in the study.

**Exclusion criteria:** Pregnant women who had a history of adrenal, parathyroid, or thyroid conditions, anemia, diabetes, severe mental disorders, or mental retardation, as well as those who were getting drugs which could alter the metabolism of vitamin D were excluded from participation.

**Sample size**

Pregnant women with good and bad pregnancy outcomes were assumed to have 25-OH-VitD of 28.46±2.7 versus 23.02±3.67 ng/ml By using online Open Epi program with a 95% confidence interval and 80% power a sample of 284 pregnant women included.

**Sample technique.**

To choose the study sample, a systemic random sample technique was applied. We chose a day at random for the outpatient clinic of the Obstetric and Gynecologic Department. We then randomly selected the first patient, and we continued selecting patients every three consecutive visits to the clinic until the study sample was completed.

**Data collection tools**

Socio-demographic details were evaluated using the Fahmy et al. questionnaire.

Obstetric and gynecological history was assessed by reporting the most recent menstrual cycle, gravidity, parity, gestational age, various pregnancy complications (preeclampsia and hypertension), and the mode of delivery. Additionally, information on fetuses and newborns was gathered, including information on intrauterine growth restriction, neonatal birth weight, APGAR score, and the existence of congenital defects were collected.
Full clinical examination including height, weight, BMI and blood pressure measuring in addition to hemoglobin A 1c (HbA1C) and blood sugar level (fasting and postprandial blood glucose levels were done for all participants.

Every patient's Vit D level serum 25-OH had been assessed in Zagazig University Hospitals laboratory According to the Brazilian Society of Endocrinology and Metabology's guidelines, which are characterized as adequate at or more than (75 nmol/L) 30 ng/mL, insufficiency among 20 along with 29 ng/mL (50 and 74 nmol/L), and deficiency at or less than (50 nmol/L) 20 ng/mL, vitamin D levels > 100 ng/mL were regarded as more than normal, we used the commonest values of vitamin D, which are utilized in most researches.

Vitamin D assessment was done firstly at the third trimester and the women were followed throughout the pregnancy. Fetal assessment was done through US. Fetal delivery and neonatal outcome were evaluated in the form Apgar score and need for admission to NICU.

Factors related to vitamin D level were assessed as kind of dressing, multivitamins use, consumption of dairy products and milk, sunlight exposure (between 10 AM and 3 PM), Consuming dairy products, such as cheese, butter, and yoghurt, that contain at least 200 milliliters of milk Consuming milk four to seven days a week was considered "sufficient," while consuming dairy items thrice each week or as fewer had been considered "in-sufficient". 12

Pregnancy-related difficulties, fetal weight, Apgar rating, gender of the newborn, as well the existence of birth defects have been among obstetric and neonatal data that were documented.

Menstrual dating accustomed to determine the gestational age at delivery, and ultrasound estimates were used for women whose dates were missed or unclear. Through early ultrasound or the last menstrual cycle, the estimated date of delivery was established.

We defined gestational hypertension as a high blood pressure readings that emerges after the twentieth week of pregnancy and is 140/90 mmHg or greater. A urine dipstick test result of ≥2+ or more than 300 milligrammes of protein detected in a sample collected over a period of 24 hours was classified as proteinuria. Gestational hypertension plus proteinuria was the definition of pre-eclampsia. 13 To diagnose gestational diabetes mellitus, or GDM, two steps were taken. An oral glucose tolerance test of 50 grammes was followed by an hour later by the measurement of plasma glucose. In order to confirm the diagnosis of GDM, a three-hour oral glucose tolerance test involving 100 grammes is performed in cases when the plasma glucose value is 135 mg/dL or higher 14.
Stillbirth is the term used to describe a fetus that dies beyond 20 weeks of pregnancy \(^{15}\). If a baby's birth weight was less than the tenth percentile of their gestational age, they were classified as small for gestational age (SGA). \(^{16}\)

**Follow up:** Vitamin D assessment was done firstly at the third trimester and the women were followed throughout the rest of pregnancy period till delivery. Fetal assessment was done through US. Neonatal outcome were evaluated in the form Apgar score and need for admission to NICU.

**Administrative Design and Ethical Aspects**

The protocol was approved by the Institutional Review Board (IRB) No. [IRB#1033-22-1-2023]. We got a formal letter of authorization from the obstetrics and gynecology department. Before beginning the research, participants were told about the nature and goals of the study, and their verbal consent was obtained. Every participant's information was kept private. The involvement of expectant mothers was entirely voluntary, anonymous, and consensual.

**Statistical analysis**

The statistical package for the social sciences, version 25 (Armonk, NY, USA), developed by IBM Corp., was utilized to analyze the information. **The Kolmogorov-Smirnov test is used to test the normality of data.** The normally distributed quantitative data were expressed using the mean, standard deviations (SD), and non-normally distributed data were expressed as median and interquartile range. The absolute frequencies and percentages of the categorical variables were used to indicate them. The Chi square test and categorical variables were used to compare the research groups. For quantitative data, the Mann-Witnney U test and the independent T-test were employed. The two-sided P value, 95% CI, and relative risk were provided as the outcomes for the qualitative components. Using Pearson's correlation analysis and binary logistic regression, the risk factors for vitamin D insufficiency were found. P values below 0.05 were considered statistically significant, and P values below 0.001 were considered extremely significant.

**Results**

The study included 284 pregnant females in the third trimester, ages ranging from 18 to 35, with a mean age of 26.41±4.94 and a mean BMI of 22.19±2.30, were included in this cohort study. In terms of education, approximately 35.6% of women could read and write or were illiterate, 25.4% finished their primary education or preparatory school, 21.8% finished their secondary education, and 17.3% completed a high degree. The majority of cases' residences (68.3%) were in urban areas and 70.4% reported that their income was insufficient. **Obstetric history revealed that median (IQR) of abortions was 0 (0-1), parity was 1(0-2), and the average mean gravidity was 2.83±1.91.** (Table 1)

As illustrated in table (2), about thirty-three percent of cases exposed to the sun. The majority of cases (70.4%) wore clothing that was covered, while only 29.6% of cases were uncovered. Sixty-seven percent
of cases (62.3%) received insufficient dairy products, and only 37.7% of cases received enough.

Vitamin D3 concentration median (IQR) was 23 (11-57) (ng/ml). About 39.8% had a vitamin D deficiency, (22.9%) had an inadequate level, 34.1% had an adequate level, and only 3.2% had a level above normal (table 2).

Between 37 and 40 weeks, the gestational age's mean at delivery, was 40.05±1.59 week. The mean fetal birth weight was 3.49±0.72 kg. The average Apgar score was 7.07±1.47 in one minute and 8.89±1.79 at five minutes. (Table 3)

In terms of the pregnancy outcomes, 19% had IDA, 5.3% had SGA, 4.9% had GDM, 4.6% each for HTN and CFM, and 4.2% had PET. More than half of the cases (58.5%) had no diseases associated with pregnancy. Only 16.5% of newborns were admitted to intensive care unit (NICU). The majority of cases (74.6%) had delivered by cesarean section. (Table 3)

When comparing vitamin D concentration to sun exposure, residence, dressing style, dairy product consumption, occupation, and use of multivitamins, A statistically significant difference (p<0.05) was observed. Women who wore clothing that was covered up had much lower vitamin D levels than women who wore clothing that was uncovered. According to table (4), women who worked outside and were exposed to the sun also had considerably higher amounts of vitamin D compared to women who worked indoors or had less exposure to the sun.

Women who took multivitamins, lived in rural areas, and consumed more dairy products were discovered to have significantly higher Vit. D levels than women who did not take multivitamins, lived in cities, and consumed fewer dairy products as illustrated in table (4).

Table (5) demonstrates that higher prevalence of vit. D level (≥ 20 ng/mL) was found among majority of women (95.5%) who were exposed to the sun, (92.9%) who dressed uncovered, (86.9%) who took multivitamins, (74.4%) of rural residents, (94.7%) of women who worked outside, and (88.8%) of women who consumed enough dairy products. In contrast, lack of vitamin D was more prevalent in urban residents, indoor-occupied, sun-exposed, covered-style dressing, 14.9 (6.2-35.9), 6.9 (3.5-13.7), and 10.5 (5.4-20.6), respectively. However, when comparing the relationship between BMI, maternal age, gravidity, gestational age at serum collection, and parity, there was no significant difference (p>0.05).

According to Table 6, there was no significant relation detected between vitamin D level and both fetal and neonatal outcomes in the form of fetal complications as small foe gestational age, fetal anomalies, IUFD, fetal birth weight and neonatal complications as needed admission to the NICU, gestational age at delivery, and Apgar scores at 1 and 5 minutes (p>0.05).

However, between women with and without vit D deficiency, there was a statistically significant difference in the incidence of HTN and pre-eclampsia: 5.4 (1.4-20.2) and 7.3 (1.5-34.5) times more common in the deficient group than the latter.
Table 7 shows potential risk factors for vitamin D3 levels as determined by binary logistic regression analysis. It was found that sun exposure and outdoor occupations were the most strongly related with vitamin D3 levels, while dressing style, use of multivitamins, place of residence, and consumption of dairy products were not significant predictors.

The correlation between vitamin D3 and various obstetric history and pregnancy outcome parameters was not statistically significant as shown in table (8)

### Table (1): demographic characteristic and obstetric history of the studied group (n=284):

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group (n=284)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>26.41±4.94 (18-35)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>22.19±2.30 (18-25)</td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>2.83±1.91 (1-11)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>No of abortions</td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0 (0-1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate, read and</td>
<td>101</td>
<td>35.6</td>
</tr>
<tr>
<td>write</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary, preparatory</td>
<td>72</td>
<td>25.4</td>
</tr>
<tr>
<td>secondary</td>
<td>62</td>
<td>21.8</td>
</tr>
<tr>
<td>High education</td>
<td>49</td>
<td>17.3</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>194</td>
<td>68.3</td>
</tr>
<tr>
<td>Rural</td>
<td>90</td>
<td>31.7</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>189</td>
<td>66.5</td>
</tr>
<tr>
<td>Outdoor</td>
<td>95</td>
<td>33.5</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not enough</td>
<td>200</td>
<td>70.4</td>
</tr>
<tr>
<td>Enough</td>
<td>83</td>
<td>29.2</td>
</tr>
<tr>
<td>Enough and more</td>
<td>1</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table (2): Vitamin D level and factors related to vitamin D deficiency among the studied group (n=284):

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group (n=284)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin D3 (ng/mL) Median (IQR)</td>
<td>23 (11-57)</td>
</tr>
<tr>
<td>Category</td>
<td>No.</td>
</tr>
<tr>
<td>Sun exposure</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>195</td>
</tr>
<tr>
<td>Yes</td>
<td>89</td>
</tr>
<tr>
<td>Dressing style</td>
<td></td>
</tr>
<tr>
<td>Covered</td>
<td>200</td>
</tr>
<tr>
<td>Uncovered</td>
<td>84</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>200</td>
</tr>
<tr>
<td>Yes</td>
<td>84</td>
</tr>
<tr>
<td>Consumption of dairy products</td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>177</td>
</tr>
<tr>
<td>Sufficient</td>
<td>107</td>
</tr>
<tr>
<td>Vit. D deficiency</td>
<td></td>
</tr>
<tr>
<td>Deficient &lt;20 ng/mL</td>
<td>113</td>
</tr>
<tr>
<td>Not deficient ≥20 ng/mL</td>
<td>171</td>
</tr>
<tr>
<td>Vit. D levels</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>113</td>
</tr>
<tr>
<td>Insufficient</td>
<td>65</td>
</tr>
<tr>
<td>Sufficient</td>
<td>97</td>
</tr>
<tr>
<td>More than normal</td>
<td>9</td>
</tr>
</tbody>
</table>
Table (3): gestational age, birth weight, Apgar score and pregnancy outcome of the studied group (n=284):

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group (n=284)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Gestational age at serum collection (weeks)</td>
<td>38.39±1.14</td>
</tr>
<tr>
<td></td>
<td>(37-40)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>40.05±1.59</td>
</tr>
<tr>
<td></td>
<td>(37-44)</td>
</tr>
<tr>
<td>Fetal birth weight (Kg)</td>
<td>3.49±0.72</td>
</tr>
<tr>
<td></td>
<td>(2-5.5)</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>7.07±1.47</td>
</tr>
<tr>
<td></td>
<td>(0-10)</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>8.89±1.79</td>
</tr>
<tr>
<td></td>
<td>(0-10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The pregnancy outcomes</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseases with pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDM</td>
<td>14</td>
<td>4.9</td>
</tr>
<tr>
<td>HTN</td>
<td>13</td>
<td>4.6</td>
</tr>
<tr>
<td>IDA</td>
<td>54</td>
<td>19.0</td>
</tr>
<tr>
<td>PET</td>
<td>11</td>
<td>3.9</td>
</tr>
<tr>
<td>Fetal outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFM</td>
<td>13</td>
<td>4.6</td>
</tr>
<tr>
<td>IUFD</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>SGA</td>
<td>15</td>
<td>5.3</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD</td>
<td>72</td>
<td>25.4</td>
</tr>
<tr>
<td>CS</td>
<td>212</td>
<td>74.6</td>
</tr>
<tr>
<td>Admission to NICU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>16.2</td>
</tr>
<tr>
<td>No</td>
<td>238</td>
<td>83.8</td>
</tr>
</tbody>
</table>

CFM= Congenital Fetal Malformation, GDM= Gestational diabetes mellitus, IDA= Iron deficiency anemia, IUFD= Intrauterine fetal demise, PET= Pre-eclampsia, SGA= Small for gestational age, VD= vaginal delivery, CS= cesarean section.
Table (4): comparing Vitamin D level and basic characteristics within the studied group:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin D concentration</th>
<th>Test (z)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17 (8-27)</td>
<td>-9.940</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Yes</td>
<td>67 (28.5-86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dressing style</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>covered</td>
<td>18 (8-25)</td>
<td>-10.322</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>uncovered</td>
<td>67.5 (47.25-84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamin use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (8-28)</td>
<td>-7.995</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Yes</td>
<td>60.5 (27.5-79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption of dairy products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16 (8-24)</td>
<td>-9.596</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Yes</td>
<td>60 (29-78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>21.5 (9-43.25)</td>
<td>-4.507</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Rural</td>
<td>46.5 (18.75-78.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>indoor</td>
<td>16 (7.5-24)</td>
<td>-10.733</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>outdoor</td>
<td>61 (44-78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>24 (13.25-55)</td>
<td>-0.275</td>
<td>0.783</td>
</tr>
<tr>
<td>VD</td>
<td>23 (10-57.75)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data was presented as Median (IQR), z = Mann-Whitney Test. ** statistically highly significant (p-value<0.001)
Table (5): Relation between Vitamin D sufficiency and basic characteristics of the studied group:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vit. D Deficient &lt;20 N=113 Mean ±SD</th>
<th>Vit. D not deficient ≥20 N=171 Mean ±SD</th>
<th>Test (t)</th>
<th>P value</th>
<th>X²</th>
<th>P value</th>
<th>RR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.26±5.05</td>
<td>26.14±4.89</td>
<td>1.645</td>
<td>0.101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>22.19±2.25</td>
<td>22.19±2.32</td>
<td>0.007</td>
<td>0.994</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.87±1.76</td>
<td>2.81±1.96</td>
<td>0.210</td>
<td>0.833</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1.39±1.63</td>
<td>1.44±1.78</td>
<td>-0.210</td>
<td>0.834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gestational age at serum collection (weeks)</td>
<td>38.48±1.16</td>
<td>38.36±1.14</td>
<td>0.732</td>
<td>0.465</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vit. D Deficient &lt;20 N=113 %</th>
<th>Vit. D not deficient ≥20 N=171 %</th>
<th>X²</th>
<th>P value</th>
<th>RR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Income</td>
<td>Not enough</td>
<td>80</td>
<td>40.0</td>
<td>0.01</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Enough</td>
<td>33</td>
<td>39.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>90</td>
<td>46.4</td>
<td>11.1</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>23</td>
<td>25.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occupation</td>
<td>Indoor</td>
<td>108</td>
<td>57.1</td>
<td>71.01</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Outdoor</td>
<td>5</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun exposure</td>
<td>No</td>
<td>109</td>
<td>55.9</td>
<td>67.40</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dressing style</td>
<td>Covered</td>
<td>107</td>
<td>53.5</td>
<td>53.1</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Uncovered</td>
<td>6</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamin use</td>
<td>No</td>
<td>102</td>
<td>51.0</td>
<td>35.5</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>11</td>
<td>13.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption of dairy products</td>
<td>Insufficient</td>
<td>101</td>
<td>57.1</td>
<td>58.5</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Sufficient</td>
<td>12</td>
<td>11.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(X²) Chi-Square Tests, (t) Independent Samples Test. ** statistically highly significant (p-value<0.001)
Table (6): Relation between Vitamin D sufficiency, Obstetric history and pregnancy outcome of the studied group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vit. D Deficient &lt;20</th>
<th>Vit. D not deficient ≥20</th>
<th>Test (t)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=113 Mean ±SD</td>
<td>N=171 Mean ±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.96±1.65</td>
<td>40.07±1.57</td>
<td>-0.536</td>
<td>0.593</td>
</tr>
<tr>
<td>Fetal birth weight (Kg)</td>
<td>3.37±0.76</td>
<td>3.53±0.7</td>
<td>-1.595</td>
<td>0.112</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>7.04±1.05</td>
<td>7.08±1.58</td>
<td>-0.198</td>
<td>0.843</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>8.94±1.38</td>
<td>8.88±1.9</td>
<td>0.254</td>
<td>0.800</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vit. D Deficient &lt;20</th>
<th>Vit. D not deficient ≥20</th>
<th>X²</th>
<th>P value</th>
<th>RR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=113 %</td>
<td>N=171 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases with pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFM</td>
<td>110 97.3</td>
<td>161 94.2</td>
<td>1.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>GDM</td>
<td>108 95.6</td>
<td>162 94.7</td>
<td>0.1</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>HTN</td>
<td>103 91.2</td>
<td>168 98.2</td>
<td>7.8</td>
<td>0.005*</td>
<td>5.4</td>
</tr>
<tr>
<td>IDA</td>
<td>88 77.9</td>
<td>142 83.0</td>
<td>1.2</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
<td>PET</td>
<td>104 92.0</td>
<td>169 98.8</td>
<td>8.4</td>
<td>0.004*</td>
<td>7.3</td>
</tr>
<tr>
<td>Fetal outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFM</td>
<td>110 97.3</td>
<td>161 94.2</td>
<td>1.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>IUFD</td>
<td>112 99.1</td>
<td>168 98.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>SGA</td>
<td>109 96.5</td>
<td>160 93.6</td>
<td>1.14</td>
<td>0.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>89 42.0</td>
<td>123 58.0</td>
<td>1.67</td>
<td>0.19</td>
<td>1.4</td>
</tr>
<tr>
<td>VD</td>
<td>24 33.3</td>
<td>66.7 48</td>
<td></td>
<td></td>
<td>(0.83-2.5)</td>
</tr>
<tr>
<td>Admission to NICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 14.2</td>
<td>30 17.5</td>
<td>0.57</td>
<td>0.44</td>
<td>0.78(0.41-1.5)</td>
</tr>
<tr>
<td>No</td>
<td>97 85.8</td>
<td>141 82.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(X²) Chi-Square Tests, (t) Independent Samples Test. * statistically significant (p-value≤0.05)

CFM= Congenital Fetal Malformation, GDM= Gestational diabetes mellitus, IDA= Iron deficiency anemia, IUFD= Intrauterine fetal demise, PET= Pre-eclampsia, SGA= Small for gestational age, VD= vaginal delivery, CS= cesarean section.
Table (7): Logistic regression analysis of factors associated with serum 25(OH)D status

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>Sig.</th>
<th>Exp(B)</th>
<th>95% C.I.for EXP(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun exposure</td>
<td>4.197</td>
<td>1.113</td>
<td>14.221</td>
<td><strong>0.000</strong></td>
<td>66.474</td>
<td>7.505 - 588.775</td>
</tr>
<tr>
<td>Dressing style</td>
<td>-1.071</td>
<td>1.215</td>
<td>0.777</td>
<td>0.378</td>
<td>0.343</td>
<td>0.032 - 3.707</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td>0.700</td>
<td>0.902</td>
<td>0.602</td>
<td>0.438</td>
<td>2.014</td>
<td>0.344 - 11.811</td>
</tr>
<tr>
<td>Consumption of dairy products</td>
<td>-0.097</td>
<td>0.790</td>
<td>0.015</td>
<td>0.902</td>
<td>0.908</td>
<td>0.193 - 4.269</td>
</tr>
<tr>
<td>Residence</td>
<td>-0.891</td>
<td>0.469</td>
<td>3.609</td>
<td>0.057</td>
<td>0.410</td>
<td>0.163 - 1.029</td>
</tr>
<tr>
<td>Occupation</td>
<td>3.344</td>
<td>1.205</td>
<td>7.706</td>
<td><strong>0.006</strong></td>
<td>28.339</td>
<td>2.673 - 300.480</td>
</tr>
</tbody>
</table>

B=regression coefficient, S.E=standard error, Sig=significance, Exp(B)=R.R and C.I=confidence interval.

* Statistically significant (p-value ≤ 0.05) and ** statistically highly significant (p-value < 0.001)

Table (8): Correlation between vitamin D3 and different obstetric history and pregnancy outcome parameters:

<table>
<thead>
<tr>
<th>Vitamin D3 (ng/MI)</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.019</td>
<td>0.744</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.010</td>
<td>0.868</td>
</tr>
<tr>
<td>Parity</td>
<td>0.043</td>
<td>0.472</td>
</tr>
<tr>
<td>No. of abortions</td>
<td>-0.027</td>
<td>0.656</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>0.036</td>
<td>0.546</td>
</tr>
<tr>
<td>Gestational age at serum collection (weeks)</td>
<td>-0.007</td>
<td>0.901</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>0.031</td>
<td>0.608</td>
</tr>
<tr>
<td>Fetal birth weight (Kg)</td>
<td>0.071</td>
<td>0.232</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>0.092</td>
<td>0.120</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>0.034</td>
<td>0.569</td>
</tr>
</tbody>
</table>

P= Sig. (2-tailed), r= Correlation Coefficient  spearman correlation  sig <0.05*
Discussion

There is evidence suggesting the connection between maternal health outcomes and vitamin D levels has long been fascinating.\textsuperscript{17}

In recent years, there has been an increased concern on the effects of maternal vitamin D deficiency and how it impacts pregnancy. It is believed that vitamin D deficiency affects pregnant females often, and low levels have been linked to negative pregnancy outcomes.\textsuperscript{18}

A recent systematic review indicates not enough levels of vitamin D in mothers increase the danger of pre-eclampsia, gestational diabetes, early labor, and other complications during pregnancy.\textsuperscript{19}

There are variations in the vitamin D levels of expecting mothers throughout the world as a result of factors such as lifestyle, BMI, skin pigmentation, and the quantity of sunlight exposure. People who have darker skin tones and have less solar exposure are more susceptible to deficiencies.\textsuperscript{20} Taking supplements can also significantly improve a pregnant woman's vitamin D status\textsuperscript{21}.

The results of this investigation show that the frequency of vitamin D insufficiency in expectant mothers is approximately 39.8%; of our cases, 22.9 percent have inadequate vitamin D levels, 34.1 percent have sufficient vitamin D levels, and only 3.2% have higher than normal levels of vitamin D. Our findings suggest that less exposure to sun, wearing clothing that covers up, living in an urban area, working indoors, and consuming fewer dairy products and multivitamins are the cause of low vitamin D levels.

According to a study by Ates et al.\textsuperscript{22} the percentage of pregnant women with vitamin D deficiency for severe (<10 ng/mL), moderate (10-19 ng/mL), and mild (20-29 ng/mL) instances were 45.9% (n = 105), 36.2% (n = 83), and 13.5% (n = 31), respectively. The proportion of pregnancies with sufficient serum 25(OH)D levels (>30 ng/mL) was only 4.4%. Their findings, which were consistent with ours, indicate that lower multivitamin use and less exposure to sunshine due to dress codes are likely the main causes of the higher occurrence of vitamin D deficiency in expecting moms.

Systematic review among South Asian has previously reported increased incidence of severe vitamin D insufficiency. Previous research has demonstrated that, for cultural and religious factors, the wearing of covered clothing may have an actual effect on vitamin D deficiency's frequency in Muslims\textsuperscript{23}.

According to our research, women with and without vitamin D deficiency have statistically different rates of hypertension and preeclampsia, with the former occurring 5.4 (1.4–20.2) and the latter 7.3 (1.5–34.5) times more frequently than the latter. Women with 25(OH)D < 20 ng/mL in the late mid-trimester (24–26 weeks of gestation) had a five-fold increased risk of preeclampsia; this risk did not increase throughout the first trimester (12–18 weeks of gestation). Unlike our research, Magnusdottir, et al. observed that women with 25 (OH) D were twice as likely to develop GDM.\textsuperscript{24}

Low vitamin D levels did not appear to be associated with the onset of GDM. On the other hand, to our research, Magnusdottir, et al. reported that Women who had a 25(OH) D < 29.4 at 15–18 weeks
gestation were twice as likely to develop gestational diabetes mellitus \(^{24}\). Ates et al. found no correlation between the development of GDM and low levels of vitamin D in the first trimester, which is consistent with our findings\(^{22}\).

A further investigation on 248 pregnant women (158 healthy controls and 90 with GDM) was conducted by Luo, et al. didn’t find association between the risk of GDM during the initial trimester and 25(OH) vit D values \(^{25}\). On the other hand, Zhang et al. discovered that in a study involving 953 pregnant females, women with GDM had significantly lower 25 (OH) vitamin D concentrations when compared to healthy controls. Furthermore, this study's findings showed that pregnant women who experienced a vitamin D shortage during their first pregnancy were more likely to develop gestational diabetes mellitus (GDM) \(^{17}\).

Furthermore, a systematic review found that a lower level of vitamin D was linked to a higher risk of GDM in a study involving 1848 patients\(^{26}\).

A noteworthy association was also noted by Pham, et al. between an increased risk of GDM and a vitamin D deficiency. In this study, one thousand forty-seven pregnant women were enrolled. According to data, Subjects with vitamin D insufficiency did not exhibit a substantially higher incidence of GDM compared to those with vitamin D deficiency \(^{27}\).

No relation was found in our research between the vitamin D levels and the delivery method, either vaginal or cesarean. Our findings concur with those of Ates et al., who found no relationship between the delivery method and vitamin D concentration\(^{22}\).

While the findings disagree with those of Amiri et al., who found that women presented with vitamin D deficiency at delivery had a higher probability of undergoing caesarean section compared to those who presented with normal quantities of this hormone \(^{28}\).

Merugu, 29 discovered that women whose 25(OH)D levels were less than 15 ng/mL at the time of delivery had an almost four-fold higher rate of primary caesarean sections. But results contradict those of two other studies: Women with 25(OH)D levels below 12 ng/mL had a higher risk of caesarean delivery, according to Zhao, et al. 30

**Conclusion:** Late pregnancy was found to have a high prevalence of vitamin D deficiency, which was linked to factors such as residence, sun exposure, employment, usage of multivitamins, and dress code. Preeclampsia and hypertension are the two negative pregnancy outcomes that have been linked to low vitamin D levels. No significant relation was detected between vitamin D level and both fetal and neonatal outcomes.

**Strengths and limitations:**
Application of the gold-standard technique for determining 25(OH) D levels along with a prospective design is considered two of the strengths of study.
On the other hand, the limited number of participants who experienced an unfavorable pregnancy outcome prevents a reliable conclusion regarding the connection between vitamin D status as well unfavorable pregnancy outcomes. Another drawback of our research is that we only assessed vitamin D amounts during a pregnancy's third trimester, neglecting to consider the potential relation between vit D levels and pregnancy outcomes during the 1st and 2nd trimesters.

Acknowledgments

We sincerely thank each and every patient who agreed to take part in our research. We also extend our gratitude to all of the staff members from the departments of Clinical Pathology, Community, Family, and Gynecology & Obstetrics who assisted us in finishing this work.

Conflict of interest

Regarding this research, each author states that there are no potential conflicts of interest. The final manuscript as submitted was approved by all authors, who also pledged to take responsibility for every facet of the job.

Funding: self-funding

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