Hypovitaminosis D in Gestational Diabetes Mellitus: The Impact of Vitamin D Status on Insulin Resistance, Parathyroid Hormone, and Perinatal Outcome

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Abstract

Objective: to evaluate serum 25-hydroxyvitamin D (25OHD) level in pregnant women who developed gestational diabetes mellitus (GDM) in comparison to healthy pregnant women, and to investigate the relationship between maternal hypovitaminosis D and insulin resistance, parathyroid hormone, and perinatal outcome.

Methods: One hundred and sixty pregnant women between 24-28 weeks gestation were recruited. Group (A) 80 pregnant women with established diagnosis of GDM, and Group (B) 80 healthy pregnant women as control. GDM was diagnosed by 75 gm oral glucose tolerance test, and insulin resistance was measured by homeostasis model assessment of IR (HOMA-IR). 25-hydroxyvitamin D, and parathyroid hormone (PTH) were measured in all participants.

Results: 116 (72.5%) of participants had 25OHD deficiency. The mean serum 25OHD level was significantly lower in GDM group compared to control (29.7±14.6 vs. 48.4±22.5 respectively). Serum 25OHD levels showed significant negative correlation with BMI, blood glucose, HOMA-IR and PTH.

Conclusions: Vitamin D deficiency is a prevalent problem among the pregnant women and can be considered as potential risk factor for development of GDM with subsequent deleterious effects on maternal and neonatal outcome.

Keywords: Hypovitaminosis D, Gestational diabetes mellitus, Insulin resistance, Parathyroid hormone.
Introduction

Gestational diabetes mellitus (GDM) is a common metabolic disorder recognized during pregnancy, and its prevalence is increasing Up to 15% - 20%. The underlying pathophysiology of gestational diabetes is inadequate insulin secretory response and increased insulin resistance (IR), whereas insulin sensitivity or responsiveness to insulin metabolic actions is decreased. GDM is associated with adverse maternal and perinatal outcome such as polyhydramnios, macrosomia, preeclampsia, and increased risk of predominant future diabetes type II.

Vitamin D is a fat-soluble vitamin as cutaneous synthesis of vitamin D3 through exposure to ultraviolet rays (90%), or naturally present in very few foods, and available as a dietary supplement. It must undergo two hydroxylations to become active in the body. The first converts vitamin D to 25-hydroxy vitamin D (25OHD) or calcidiol in the liver, while the second hydroxylation occurs primarily in the kidney to become physiologically active form 1,25-dihydroxyvitamin D (1,25OHD3) or calcitriol, which is tightly regulated by plasma parathyroid hormone (PTH) as well as serum calcium and phosphate levels.

Vitamin D plays a major role in calcium homeostasis, and it was found to have extra skeletal functions. It also has an important role in glucose and insulin metabolism, as it increases insulin secretion through its receptors in pancreatic islet cells or by modulating the immune system. Moreover, it increases insulin sensitivity via increasing insulin receptors or enhancing receptor sensitivity to insulin, and its influence on extracellular calcium regulation. Consequently, vitamin D may affect insulin resistance and insulin secretions which are two pathogenic factors involved in type II diabetes mellitus development.

PTH is secreted by parathyroid gland when serum calcium is below 4.4 mg/dL. Then, PTH binds to their PTH-receptors in kidney and stimulates CYP27B1 renal expression, which conduces to a higher production of calcitriol from available calcidiol. Calcitriol will be in charge of increasing serum calcium levels through a higher intestinal absorption, renal reabsorption and bone resorption. So, PTH induces the synthesis of vitamin D. Hypovitaminosis D is reported to be common in the Middle East, and African countries despite ample sunshine. It is suggested to be associated with adverse health outcomes including musculoskeletal, cardiovascular, renal, metabolic, autoimmune, neurological, infectious diseases, and cancer. It has been linked with preeclampsia, gestational diabetes and small for gestational age, neonatal hypocalcaemia, and seizures. Pregnancy in general is associated with insulin resistance and hyperinsulinemia which is aggravated by maternal vitamin D status thus predisposing to development of gestational diabetes. Moreover, the existence of Vitamin D receptors (VDR) within pancreatic β-cells with local production of calcitriol suggests that deficient vitamin D levels may increase risk of diabetes mellitus.

The aim of the current study is to evaluate serum 25-hydroxyvitamin D (25OHD) levels in pregnant women who developed GDM in comparison to healthy pregnant women, and to investigate the relationship between maternal hypovitaminosis D and insulin resistance, PTH, and perinatal outcome.

Patients and Methods

This observational case control study was conducted at the Department of Obstetrics and Gynecology of Ibn Sina College Hospital, Saudi Arabia, between August 2014 and July 2016. One hundred and sixty pregnant women attending the antenatal care clinic were recruited at the time of antenatal screening for GDM at 24-28 weeks. Eighty pregnant women fulfilled the inclusion criteria with established diagnosis of GDM were classified as (group A). Age and gestational age matched eighty healthy pregnant women were included as controls (Group B). Exclusion criteria include, pre-existing type I or type II diabetes, previous history of gestational diabetes, metabolic bone disease, impaired renal or liver functions,
medical disorders or any pregnancy induced complications as preeclampsia, any medications interact with vitamin D metabolism such as steroids, and statins, history of vitamin D supplementation. This study was approved by the Hospital Research Ethics Committee and has been performed in accordance with the ethical standards as in Declaration of Helsinki (1964) and its later amendments, and a written informed consent was obtained from each participant. All participants were briefed about the nature of the study, and a detailed obstetrical, medical, and family history was taken. After that, thorough clinical examination and anthropometric measurements were done. The pre-pregnancy body mass index (BMI) was calculated using the formula: weight (kg)/ height (m²), and patients were classified according to BMI using the World Health Organization ranges.

For diagnosis of gestational diabetes one-step approach was used. A 75 gm oral glucose tolerance test (75g OGTT) was performed in all participants at 24-28 weeks gestation. GDM defined if at least one of the following diagnostic criteria was met: fasting: ≥92 mg/dL (5.1 mmol/L), 1 h: ≥180 mg/dL (10.0 mmol/L), 2 h: ≥153 mg/dL (8.5 mmol/L); according to the guidelines of the international association of diabetes and pregnancy study groups (IADPSG) 2010. 7 Maternal fasting blood samples were obtained from the ante-cubital vein under complete aseptic conditions from all subjects and divided into two parts. The first part was collected in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis. Plasma was separated by centrifugation and used for estimation of glucose by glucose oxidase method, (Vitros 350 Chemistry System, Ortho Clinical Diagnostics, USA). The second part was collected in a plain tube and left to clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000g. Freshly prepared serum was stored at -20°C till estimation of fasting serum insulin (FSI), 2 hour post prandial insulin (2h PPI), 25OHD, and PTH. Serum insulin (SI) was measured using Abbott Architect i1000 Chemiluminescence Immunoassay, Germany. Insulin resistance (IR) was measured by homeostasis model assessment of IR (HOMA-IR) on the basis of insulin and glucose levels and according to the formula fasting serum insulin (µU/ml) x [fasting plasma glucose (mg/ml)] /405; HOMA-IR index >2 is considered abnormal. 8 Serum Vitamin 25OHD was measured using Abbott Architect i1000 Chemiluminescence Immunoassay, Germany. Vitamin D insufficiency was defined as a 25OHD concentration of 50–75 nmol/L, and Vitamin D deficiency was defined as a 25OHD concentration of <50 nmol/L and was classified as mild (25–50 nmol/L), moderate (12.5–25.0 nmol/L), and severe (<12.5 nmol/L). 9 Serum PTH levels were measured by Chemiluminescence Immunoassay (DPC Immulite 2000 Immunoassay System, USA). All women were followed up till delivery and maternal and neonatal outcome were reported.

Statistical analysis
The Data was collected and entered into the personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 20) software. Arithmetic mean, standard deviation, for categorized parameters Chi square test was used while for numerical data for more than two groups ANOVA test was used with post-hoc Tukey HSD Test. To find the association between two variables, Spearman correlation coefficient test was used. The level of significant was 0.05.

Sample size calculation
An adequate sample size is needed to estimate the population prevalence with a good precision. The sample size and should depend on the research context, including the researcher’s objectives and proposed analyses. The following formula was used to calculate the required sample size in this study;

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Results
A total of one hundred and sixty consented pregnant women at 24-28 weeks were classified into two groups, eighty participants with established diagnosis of GDM in group (A) compared to an another eighty healthy pregnant women in group (B) as controls. The demographic characteristics of the study participants are illustrated in table (1). There were no statistically significant differences between the two groups for maternal age, gravidity, and parity. The mean BMI was significantly higher in GDM group compared to control (28.7±3.9 vs. 27.5±3.3 respectively, p=0.037). The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) in GDM women showed a significant increase compared to controls (124.5±8.2 vs. 121.5±5.8, p=0.008; 77.8±7.2 vs. 75.2±6.6, p=0.032 respectively). (Table 1)

In this study, the mean fasting blood glucose (FBG) and FSI levels were significantly higher in GDM group compared to control (123.9±6.8 vs. 81.7±10.5, p=0.042; 9±3.27 vs. 7.1±2.68, p=0.009 respectively), together with significantly higher HOMA-IR in women of group (A) compared to group (B) (2.67±0.69 vs. 1.45±0.49, p=0.001). Moreover, 74 pregnant women had HOMA-IR score of >2; 62 (77.5%) women in GDM group and only 12 (15%) women in control group which was also statistically significant (p=0.009). (Table 2)

A regard vitamin D, 116 (72.5%) of the study participants had 25OHD deficiency (<50nmol/L), the frequency of such deficiency was significantly higher in GDM group 70 (87.5%) compared to controls 46 (57.5%) (p=0.0001), while only 19(11, 9%) of the study participants had 25OHD sufficiency (>75nmol/L). The mean serum 25OHD level was significantly lower in GDM group compared to normal pregnant women (29.7±14.6 vs. 48.4±22.5 respectively, p=0.001). Moreover, the same results were also observed in the different three categories of 25OHD status (sufficiency, insufficiency, deficiency) between both groups. (Table 3)

When 25OHD deficiency was sub classified into three degrees (mild, moderate, severe), it was realized that, although the frequency of pregnant women distribution among degrees of 25OHD deficiency was statistically insignificant between both groups (p>0.05), the estimated mean serum 25OHD levels were significantly lower in GDM group compared to healthy pregnant women in all three degrees of 25OHD deficiency. Furthermore, the current study showed that, the mean serum PTH level was significantly higher in GDM group compared to controls (33.35±11 vs. 27.8±9.4 respectively, p=0.001). (Table 3)

When the whole study population was taken into consideration, HOMA-IR showed significant positive correlation with age (r=0.204, p=0.010), BMI (r=0.314, p=0.0007), SBP (r=0.220, p=0.005), DBP (r=0.308, p=0.0008), FBG (r=0.651, p=0.0003), post prandial blood glucose (PPBG)(r=0.642, p=0.), and FSI (r=0.577, p=0.0007), while it showed significant negative correlation with serum 25OHD (r=-0.437, p=0.0005), and insignificant negative correlation with PTH levels(r=-0.103, p= >0.05). On the other hand, serum 25OHD level showed significant negative correlation with BMI (r=-0.652, p=0.0002),FBG (r=-0.358, p=0.001),PPBG(r=-0.381, p=0.0009), FSI (r=-0.253, p=0.001), HOMA-IR (r=-0.437, p= 0.0005), and PTH (r=-0.192, p= 0.015), while it showed insignificant negative correlation with age, SBP and DBP. (Table 4)

Regarding the perinatal outcome, Eighty-three women (51.9%) had normal vaginal delivery, 22 women (13.8%) had elective cesarean section.
(CS), and 55 women (34.3%) had emergency CS with significantly higher frequency of CS (p=0.022) in GDM group in comparison to control group. The neonates required NICU admission, and the mean Apgar score determined at 1-min and 5-min showed statistically insignificant difference (p>0.05) between newborns of both groups. (Table 5) HOMA-IR showed significant negative correlation with maternal and neonatal outcome (Table6), while serum 25OHD showed significant positive correlation with the same parameters in both groups. (Table7)

**Table (1): Demographic data and clinical characteristic of Patients in group (A), and (B).**

| Variables            | GDM group (A) (n=80) | Control group (B) (n=80) | P value |
|----------------------|----------------------|--------------------------|---------|
| Age group (years)    |                      |                          |         |
| <20                  | 0                    | 1 (1.3%)                 | >0.05   |
| 20-25                | 15 (18.8%)           | 12 (15%)                 |         |
| 25-30                | 51 (63.8%)           | 60 (75%)                 |         |
| >30                  | 14 (17.4%)           | 7 (8.7%)                 | >0.05   |
| Mean age             | 28.2±2.3             | 27.8±2.4                 |         |
| Gravidity            |                      |                          | >0.05   |
| Primigravida         | 18 (22.4%)           | 26 (32.5%)               |         |
| Multigravida         |                      |                          |         |
| 2                    | 25 (31.3%)           | 23 (28.7%)               |         |
| 3                    | 24 (30%)             | 22 (27.5%)               |         |
| >3                   | 13 (16.3%)           | 9 (11.3%)                |         |
| Mean gravidity       | 2.4±1                | 2.2±1                    | >0.05   |
| Mean parity          | 1.4±1                | 1.2±1                    | >0.05   |
| Weight (kg)          | 83.1±11.4            | 79.3±9.5                 | 0.022   |
| Height (cm)          | 170±3.2              | 169.7±3.5                | >0.05   |
| Pre-pregnancy BMI (kg/m²) |          |                          | >0.05   |
| Average Wt           | 22 (27.4%)           | 29 (36.3%)               |         |
| Over Wt              | 31 (38.8%)           | 34 (42.5%)               |         |
| Obese                | 27 (33.8%)           | 17 (21.2%)               |         |
| Mean                 | 28.7±3.9             | 27.5±3.3                 | 0.037   |
| SBP                  | 124.5±8.2            | 121.5±5.8                | 0.008   |
| DBP                  | 77.8±7.2             | 75.2±6.6                 | 0.032   |

Data presented as n (%) or mean±SD, P <0.05: indicates significant difference.

GDM: Gestational diabetes mellitus, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

**Table (2): Glucose homeostasis and HOMA-IR of Patients in group (A), and (B).**

| Variables         | GDM group(A) (n=80) | Control group(B) (n=80) | P value |
|-------------------|---------------------|-------------------------|---------|
| 75g OGTT          |                     |                         |         |
| FBG (mg/dl)       | 123.9±6.8           | 81.7±10.5               | 0.042   |
| 1-hr PPBG (mg/dl) | 229.4±24.8          | 165.2±6.4               | 0.001   |
| 2-hr PPBG (mg/dl) | 167.6±4.5           | 124.4±5.7               | 0.001   |
| SI                | 9±3.27              | 7.1±2.68                | 0.009   |
| 2-hr PPSI (µU/ml) | 64.1±23.1           | 18.7±3.7                | 0.001   |
| HOMA-IR score     | 2.67±0.69           | 1.45±0.49               | 0.001   |

Data are presented as mean±SD, P <0.05: indicates significant difference.

GDM: Gestational diabetes mellitus, 75g OGTT: 75 gm oral glucose tolerance test, FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, SI: Serum insulin, FSI: Fasting serum insulin, 2-hr PPSI: 2-hours postprandial serum insulin, HOMA-IR: Homeostasis model assessment of insulin resistance.
Table (3): Categorization and biochemical levels of 25OHD and PTH of Patients in group (A), and (B)

| Variables                           | GDM group (A) (n=80) | Control group (B) (n=80) | P value |
|-------------------------------------|----------------------|--------------------------|---------|
| Vitamin D Categories                |                      |                          |         |
| Frequency                           |                      |                          |         |
| Sufficient level                    | 3 (3.7%)             | 16 (20%)                 | 0.0001  |
| Insufficient level                  | 7 (8.8%)             | 18 (22.5%)               |         |
| Deficient level                     | 70 (87.5%)           | 46 (57.5%)               |         |
| Mean                                |                      |                          |         |
| Sufficient level                    | 76±1                 | 79.7±2.12                | 0.014   |
| Insufficient level                  | 56.7±1.8             | 64.8±4.5                 | 0.001   |
| Deficient level                     | 25±7.4               | 31.2±11                  | 0.001   |
| Serum 25OHD (nmol/L)                | 29.7±14.6            | 48.4±22.5                | 0.001   |

| Vitamin D deficiency                |                      |                          |         |
| Frequency                           |                      |                          |         |
| Mild deficiency                     | 48 (60%)             | 30 (37.5%)               | >0.05   |
| Moderate deficiency                 | 14 (17.5%)           | 11 (13.8%)               |         |
| Severe deficiency                   | 8 (10%)              | 5 (6.2%)                 |         |
| Mean                                |                      |                          |         |
| Mild deficiency                     | 29.6±2.5             | 31.1±4.1                 | 0.001   |
| Moderate deficiency                 | 18±1.5               | 19.6±2.1                 | 0.001   |
| Severe deficiency                   | 10±1.8               | 11±1.4                   | 0.005   |
| PTH (pg/ml)                         | 33.35±11             | 27.8±9.4                 | 0.001   |

Data presented as n (%) or mean±SD, P <0.05: indicates significant difference.

GDM: Gestational diabetes mellitus, PTH: Parathyroid hormone.

Table (4): Correlation coefficient "r" of HOMA-IR, and 25 OHD with anthropomorphic and biochemical data of total studied patients

| Variables   | HOMA-IR | 25OHD |
|-------------|---------|-------|
| Age         | r       | 0.204 | -0.124|
|             | p       | 0.010 | >0.05 |
| BMI         | r       | 0.314 | -0.652|
|             | p       | 0.0007| 0.0002|
| SBP         | r       | 0.220 | -0.189|
|             | p       | 0.005 | >0.05 |
| DBP         | r       | 0.308 | -0.121|
|             | p       | 0.0008| >0.05 |
| FBG         | r       | 0.651 | -0.358|
|             | p       | 0.0003| 0.001 |
| PPBG        | r       | 0.642 | -0.381|
|             | p       | 0.0004| 0.0009|
| FSI         | r       | 0.577 | -0.253|
|             | p       | 0.0007| 0.001 |
| HOMA-IR     | r       | -     | -0.437|
|             | p       | -     | 0.0005|
| 25OHD       | r       | -0.437| -     |
|             | p       | 0.0005| -     |
| PTH         | r       | -0.103| -0.192|
|             | p       | >0.05 | 0.015 |

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, SI: Serum insulin, HOMA-IR: Homeostasis model assessment of insulin resistance, PTH: Parathyroid hormone, r: Correlation, P <0.05: indicates significant difference.
Table (5): Maternal and neonatal outcome of studied patients of both groups

| Variables             | GDM group (A) (n=80) | Control group (B) (n=80) | P value  |
|-----------------------|----------------------|--------------------------|----------|
| Mode of delivery      |                      |                          |          |
| Normal vaginal delivery | 32 (40%)             | 51 (63.8%)               | 0.022    |
| CS                    |                      |                          |          |
| Emergency             |                      |                          |          |
| Prolonged labor       | 18 (22.5%)           | 9 (11.2%)                |          |
| Fetal Distress        | 16 (20%)             | 12 (15%)                 |          |
| Elective              | 14 (17.5%)           | 8 (10%)                  |          |
| Neonatal Outcome      |                      |                          |          |
| APGAR Score           |                      |                          |          |
| 1-min                 | 6.1±0.9              | 6.3±0.9                  | >0.05    |
| 5-min                 | 8.1±1.2              | 8.3±0.8                  | >0.05    |
| NICU admission        |                      |                          |          |
| Ventilation           | 5 (6.3%)             | 3 (3.8%)                 | >0.05    |
| Phototherapy          | 4 (5%)               | 3 (3.8%)                 |          |
| Sepsis                | 2 (2.5%)             | 1 (1.3%)                 |          |
| Mortality             | 2 (2.5%)             | 1 (1.3%)                 |          |
| No NICU admission     | 67 (83.7%)           | 72 (89.8%)               | >0.05    |

Data presented as n (%) or mean±SD, P <0.05: indicates significant difference.
GDM: Gestational diabetes mellitus, CS: Cesarean section, NICU: Neonatal Intensive Care Unit.

Table (6): Correlation coefficient "r" between HOMA-IR and outcome of studied patients of both groups

| Variables             | GDM group (A) (n=80) | Control group (B) (n=80) | P value  |
|-----------------------|----------------------|--------------------------|----------|
| Frequency of CS       | r 0.291 p 0.009      | -0.262 p 0.019           |          |
| APGAR score 1-min     | r -0.221 p 0.049     | -0.378 p 0.001           |          |
| APGAR score 5-min     | r -0.264 p 0.018     | -0.223 p 0.047           |          |
| NICU admission        | r 0.321 p 0.004      | 0.250 p 0.025            |          |

GDM: Gestational diabetes mellitus, HOMA-IR: Homeostasis model assessment of insulin resistance, 25OHD: 25-hydroxy vitamin D, CS: Cesarean section, NICU: Neonatal Intensive Care Unit, r: Correlation, P <0.05: indicates significant difference

Table (7): Correlation coefficient "r" between serum levels of 25OHD and outcome of studied patients of both groups

| Variables             | GDM group (A) (n=80) | Control group (B) (n=80) | P value  |
|-----------------------|----------------------|--------------------------|----------|
| Frequency of CS       | r 0.236 p 0.035      | 0.273 p 0.014            |          |
| APGAR score 1-min     | r 0.286 p 0.010      | 0.342 p 0.002            |          |
| APGAR score 5-min     | r 0.432 p 0.009      | 0.421 p 0.009            |          |
| NICU admission        | r -0.264 p 0.018     | -0.366 p 0.001           |          |

GDM: Gestational diabetes mellitus, HOMA-IR: Homeostasis model assessment of insulin resistance, 25OHD: 25-hydroxy vitamin D, CS: Cesarean section, NICU: Neonatal Intensive Care Unit, r: Correlation, P <0.05: indicates significant difference
Discussion
The prevalence of hypovitaminosis D is in growing problem among both non pregnant and pregnant women. In the current study, 141(88.1%) pregnant women of our participants had insufficient/deficient serum 25OHD level, and 116(72.5%) out of all participants had vitamin D deficiency (25OHD level<50nmol/L), and 13(8.1%) out of all participants had severe hypovitaminosis D. This prevalence is in agreement with other previous studies as AlKalbani et al\textsuperscript{10} reported 98% vitamin D deficiency among Omani pregnant women. Also, Al-Shaikh et al\textsuperscript{11} reported 86.4% of Saudi pregnant women exhibited vitamin D deficiency. Recently Karras et al\textsuperscript{12} in a systemic review of 2649 pregnant women in Mediterranean region reported that, the prevalence of vitamin D deficiency ranged from 22.7 to 90.3%.

Actually hypovitaminosis D is a global problem, thought to be related to inadequate exposure to sunlight, dietary inadequacy, increased requirements, clothing style which prevents all-day exposure to sunrays, fat malabsorption syndrome, medications enhancing the catabolism of vitamin D, and obesity which is now considered as a major contributory factor resulting in severe skeletal and non-skeletal health events for mothers and off spring since the growing fetus depends on maternal body reserves for its bone mineralization, so pregnant women are at a real risk. Vinkhuyzen et al\textsuperscript{13} reported 26% of pregnant women at midgestation, 46% of neonates were vitamin D deficient and 21% of the mother-infant pairs had persistent vitamin D deficiency.

Our study showed a significant inverse relation between 25OHD level and GDM, as it was significantly lower in GDM group compared to normal pregnant women (29.7±14.6 vs. 48.4±22.5 respectively, p=0.001), and 87.5% of GDM women had 25OHD<50 nmol versus 57.5% of controls. These results are in concordance with previous studies that documented the association between low 25OHD level and increased risk of GDM.\textsuperscript{14,15} On the contrary, other studies did not detected any significant association between 25OHD level and GDM at different trimesters of pregnancy.\textsuperscript{16,17}

The role of hypovitaminosis D in the pathogenesis of GDM is still controversial but several risk factors have been recognized, as ethnicity, family history of diabetes, advanced maternal age, and obesity. Vitamin D plays an important role in glucose homeostasis and its deficiency may alter glucose tolerance, insulin secretion and sensitivity of the target cells via direct activation of Vitamin D receptors especially in pancreatic B cells, or through its regulatory effect on plasma calcium levels, or indirectly by acting on different immune cells. Inverse association between serum 25OHD and insulin resistance have been established in several epidemiological studies. Subsequently, vitamin D supplementation during pregnancy has beneficial effects on glycaemia, insulin sensitivity, and metabolic profiles, but the appropriate intake of vitamin D in pregnancy is still debatable.

Mutlu et al\textsuperscript{18} concluded that hypovitaminosis D, insulin resistance, and gestational diabetes mellitus are interrelated with adverse maternal and perinatal outcome notably with severe type. Al-Shaikh et al\textsuperscript{11} detected a significant negative correlation between serum 25OHD levels and FBG concentrations. Also, Lacroix et al\textsuperscript{19} suggested that low levels of 25OHD at first trimester are an independent risk factor for developing GDM and associated with insulin resistance at second trimester. Moreover, Daniel and Keller\textsuperscript{20} clarified that lower levels of 25OHD were associated with an increased risk of GDM by 40% for each one standard deviation decrease in 25OHD level (odds ratio, 1.40/1 SD) [95% CI, P = 0.04]. In concordance with these studies, our data showed significant negative correlations between serum 25OHD levels and HOMA-IR, FBG, FSI and maternal BMI. Many studies reported an inverse association between vitamin D levels and BMI as Ford et al\textsuperscript{21,22}, while Grimnes et al, and McCarty et al did not detect such association.\textsuperscript{23,24}
Ultimately, these women are in a vicious cycle of sedentary lifestyle, obesity and hypovitaminosis D aggravated by getting pregnant ending in development of GDM that may progress to postpartum manifest DM. In our study, PTH levels were significantly higher in women with GDM compared to controls (33.35±11 vs. 27.8±9.4 respectively, p= 0.001), and serum 25OHD level showed significant negative correlation with PTH. Elevated PTH levels to other disorders have been shown to be associated with decreased insulin sensitivity, impaired glucose tolerance, and an increased risk of DM. Therefore, high PTH concentrations may have an additional effect on glucose tolerance during pregnancy.

In the current study, although the incidence of cesarean delivery was higher in GDM group compared to controls (60% vs. 36.2%, p= 0.022) but APGAR-1 and 5 min scores were statistically insignificant in neonates of both groups. Furthermore, there was a significant negative correlation between HOMA-IR and maternal and neonatal outcome while serum 25OHD levels showed a significant positive correlation with the same parameters. On the contrary, Mutlu et al 18 reported lower APGAR-1 and 5min scores in GDM neonates than those of normal response to OGGT but in agreement with our study they found APGAR scores were correlated negatively with insulin resistance and positively with 25OHD levels. Similarly, Reichetzeder et al 25 reported that, maternal 25OHD deficiency was independently associated with low APGAR scores 5 minutes postpartum.

Conclusion
Vitamin D deficiency is a prevalent problem among the pregnant women and can be considered as potential risk factor for development of GDM with subsequent deleterious effects on maternal and neonatal outcome. Further trials are advocated to evaluate the protective value of optimal plasma level of 25OHD on the prevalence of GDM and on pregnancy outcome. Screening pregnant women who are at risk of hypovitaminosis D is recommended and proper vitamin D supplementation should be considered.

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