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# Association of 25(OH)D levels in pregnancy with gestational diabetes mellitus

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***Abstract*---**This study evaluate the association of 25 (OH)D levels in pregnancy with GDM as a case group (n=31) and without GDM as a control group (n=31), and the association between levels of 25 (OH)D in pregnancy with OGCT 50g and OGTT 100g fasting, 1 hour, 2 hours, and 3 hours. This study is an unmatching hospital-based case-control at Dr. Soetomo and Airlangga University hospital Surabaya from February 2022 to May 2022, GDM was diagnosed according to the ADA guidelines. Vitamin D status was defined as, levels of 25 (OH)D normal (30-100 ng/mL), insufficiency (20-29.9 ng/mL), deficiency (<20 ng/mL). The mean serum 25(OH)D level was lower in case group 14.7 + 4.09 ng/mL compared to that in control group 28.9 + 9.10 ng/mL. There was a significant association between 25(OH)D levels and GDM (p=0.001). Samples with deficiency 25(OH)D 11.94 times that increases the risk of developing GDM. There was a significant association between 25(OH)D levels and OGCT 50g levels (p=0.001). There was no significant association between 25(OH)D levels with OGTT levels of 100g fasting (p=0.747), 1 hour (p=0.148), 2 hours (p=1,000), and 3 hours (p=0.469).

***Keywords*---**gestational diabetes mellitus, vitamin D (25OH)D, pregnancy.


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## Introduction

Vitamin D, a fat-soluble vitamin, acts as a pleiotropic hormone in most human tissues by regulating mineral homeostasis and various other biological functions, including effects on innate and adaptive immunity. Vitamin D also has important functions in pregnancy such as maintaining the balance of glucose metabolism, controlling inflammation and infection responses. Several studies have reported that vitamin D deficiency is also associated with an increased risk of preeclampsia, gestational diabetes mellitus, low birth weight, and premature birth (Mahmood et al., 2021).

Vitamin D status in the body is determined based on the level of 25 (OH)D (calcidiol) which is one of the metabolites of vitamin D. This is because the half-life of 25(OH)D is quite long, which is 2-3 weeks. In addition, 25(OH)D is easily examined and has the highest levels among other vitamin D metabolites, and has a strong correlation between the state of 25(OH)D deficiency and clinical symptoms. Directly or indirectly, 25(OH)D affects pancreatic -cell function and insulin secretion, 25(OH)D is also required to ensure normal levels of calcium in cell membranes that are important for insulin (Liu and Hewison, 2012). At no other time during the lifecycle is vitamin D status more important than during pregnancy as the mother is the sole source of vitamin D substrate for her developing fetus. While vitamin D status during pregnancy varies around the globe as a function of maternal sunlight exposure, degree of skin pigmentation, latitude, lifestyle, body mass index (BMI) and the intake of oral vitamin D supplements, it is clear that those with darker pigmentation and limited sunlight exposure are at greatest risk for deficiency (Pludowski et al., 2013).

GDM is a public health problem because this disease has a direct impact on the health of the mother and fetus. The impact caused by mothers with GDM is that mothers are at high risk of excess weight gain, the occurrence of preeclampsia, eclampsia, cesarean section, and cardiovascular complications to maternal death. After delivery occurs, the patient is at risk of developing type 2 diabetes or developing GDM that will recure in the future. Babies born to mothers with GDM are at high risk for macrosomia and birth trauma. In addition, infants are at high risk for developing hypoglycemia, hypocalcemia, hyperbilirubinemia, respiratory distress syndrome, polycythemia, obesity and type 2 diabetes mellitus (Alzaim, 2013). Hormonal and metabolic changes during pregnancy are causes the pregnancy to be diabetogenic, which means that GDM tends to become heavier during pregnancy and will facilitate the occurrence of various complications. Mothers with GDM approximately 1.7% can cause perinatal mortality, 4.3% birth by caesarean section surgery, 7.3% give birth to children whose birth weight is more than 4.5 kg and 23.5% can cause cases of shoulder dystocia in the birth process. Gestational diabetes mellitus occurs in about 4% of all pregnancies in the United States, and 3-5% in the United Kingdom. The prevalence of GDM in Europe is 2-6% (Coustan, 2016). While in Indonesia around 1.9-3.6%, at Dr. Soetomo hospital Surabaya, Joewono, et al in 1992 found that 12 of the 602 cases studied were diagnosed as GDM (1.99%). Joewono, et al in 2010 found that out of 75 cases, 3 were positive GDM (4%) (Joewono, 2014).

Indonesia is a country that has a tropical climate, which gets enough sunlight but there are still occurrences of vitamin D deficiency (25(OH)D), especially during pregnancy, and there is still a very limited number of studies on the effect of 25 (OH)D levels on glucose blood levels during pregnancy in Indonesia. Therefore, this study was designed to determine the relationship between 25(OH)D levels in pregnancy and the incidence of GDM.

**Method**

This study was performed with an analytical observational study design in the form of hospital-based case-control unmatching study at the department of obstetrics and gynecology of medicine Airlangga University Dr. Soetomo Hospital Surabaya from February 2022 to May 2022, with samples of pregnant women with gestational diabetes mellitus as the case group and pregnant woman with normoglycemia as the control group with a total of 62 samples, according to the inclusion criteria, pregnant women with singleton fetus, pregnant women with GDM (case), pregnant women with normoglycemia (control), gestational age > 24 weeks, and exclusion criteria pregnant women with multiple fetus, pregnant women with pregestational diabetes, and pregnant women with hypertension in pregnancy. 5 ml of blood was taken from antecubital vein. Research samples were examined for levels of OGCT 50 g and OGTT 100 g fasting, 1 hour, 2 hours and 3 hours. It examined for level of OGCT (oral glucose challenge test) with a load of 50 grams on all pregnant women > 24 weeks and 1 hour later venous plasma glucose is measured. If a value of 135 mg % is obtained, an OGTT (oral glucose tolerance test) is performed with a load of 100 grams and fasting glucose is measured at 1, 2 and 3 hours. It is said to be GDM from ADA guidelines if there are 2 or more values of blood sugar levels that exceed the value of the four examinations which include fasting blood sugar 95 (5.3), blood sugar 1 hour 180 (10.0), blood sugar 2 hours 155 (8.6), blood sugar 3 hours 140 (7.8) (ADA, 2016). Then after obtaining 31 case samples of pregnant women with GDM and 31 control samples of pregnant women with normoglycemia, all samples were examined for serum vitamin D (25(OH)D) levels.

Serum vitamin D level was measured by chemiluminescence micro particles immunoassay method (CM1A) in Abbott architect system. According to previously published criteria, vitamin D deficiency (<20ng/ml), insufficiency (20-29.9 ng/ml), normal (30-100 ng/ml). Data were analyzed by using computer software SPSS (Statistical package for social sciences, version 23) and statistical analysis was performed by Chi-squared (x2) test for the comparison of data presented in categorical scale. Non-parametric Mann-Whitney Test for the data OGCT 50g and Non-parametric Kruskal-Wallis Test for the data OGTT 100g. The $p<0.05$ was considered statistically significant. Strength of association was determined by the estimating odds ratio and their 95% confidence intervals.

**Results**

The majority of research samples included in the age group of 18-35 years that is equal to 79.0%. The youngest subject was 20 years old and the oldest was 39 years old. The proportion of the samples gestational age was greater at 20-34 weeks which was 95.2%, than the gestational age >34 weeks which was 4.8%.

Most of the samples had a Body Mass Index (BMI) which was included in the overweight class of 40.3%, followed by obesity class I 29.0%. The proportion of primiparous and multiparous samples was found to be more in multipara parity by 83.9% compared to primiparas, with the most recent education being secondary (junior high school or high school). Most of the research samples do not work, but for those who work, among others, as traders, nurses, teachers, waiters, and household assistants. The majority of samples (91.9%) consumed foods containing vitamin D at least 2 times a week. Most of the samples (85.5%) did activities outside the home/office for less than 90 minutes. The use of sunscreen and long-sleeved clothing was found in 1.6% and 79.0% of samples, respectively. More than half of the samples 56,5% had levels of 25(OH)D < 20 ng/mL (deficiency), while levels of 25(OH)D between 20-29.9 ng/mL (insufficiency) were 24.2%, and the rest had levels of The normal 25(OH)D > 30 ng/mL was 19.4%.

The age of the samples did not differ significantly between the case and control groups. In each group, the proportion of gestational age of 24-34 weeks was higher than that of gestational age >34 weeks. BMI values in the two groups were highest in the overweight group and Class I obesity in the second. The proportion of parity was almost the same in both groups where the number of parity multiparas was more than primiparas by 77.4% in GDM and 90.3% in the control group. In both groups, the majority of education was in secondary education, 80.6% in the GDM group and in the control group. The majority of GDM and control group samples were housewives. The highest 25(OH)D deficiency was found in the GDM group. While levels of 25(OH)D insufficiency were more commonly found in the control group. Normal levels of 25(OH)D were found in the control group as many as 12 samples, while in the GDM group there were no normal values for 25(OH)D levels. The majority of research samples were in a condition of vitamin D deficiency, with serum 25(OH)D levels < 20 ng/mL 35 sampels. The majority of samples with vitamin D deficiency were multipara (77.1%), secondary education (80.0%), not working (74.3%), consuming foods containing vitamin D more than 2 times a week (94.3%) , wear clothes that cover the arms and hands during activities (94.3%) and all do not wear sunscreen (100.0%). Meanwhile, in samples with vitamin D insufficiency, the composition of characteristics was almost the same, multipara (86.7%), secondary education (73.3%), not working (73.3%), consuming foods containing vitamin D more than 2 times. a week (86.7%), using clothes that cover the arms and hands when doing activities outside the home (93.3%) and all do not use sunscreen (100.0%).

Levels of 25(OH)D had the highest levels of 53.10 ng/mL in the control group and 21.30 ng/mL in the GDM group. The lowest value was 15.90 ng/mL in the control group and 6.70 ng/mL in the GDM group. Overall in the study samples, the mean serum 25(OH)D level in the control group was 28.9 + 9.10 ng/mL and GDM was 14.7 + 4.09 ng/mL. There was a significant relationship between 25(OH)D levels and the incidence of GDM (p=0.001). And if the data is grouped into levels of deficiency and non-deficiency associated with the incidence of GDM, the OR value = 11.94 with 95% CI (3.11-45.73). Which can be interpreted as a deficiency of 25(OH)D 11.94 times that increases the risk of developing GDM. From the data obtained in research samples with 25(OH)D deficiency, the value of OGCT 50g levels was higher than research samples who had 25(OH)D levels which were in insufficiency and normal levels. There was a significant relationship between 25(OH)D levels and OGCT 50g levels with (p=0.001).

The results relationship analysis between levels of 25(OH)D with fasting OGTT levels of 100g p=0.747, OGTT 100g 1 hour p=0.148, OGTT 100g 2 hours p=1,000, OGTT 100g 3 hours p=0.469. So it can be concluded that there is no significant relationship between levels of 25(OH)D with OGTT levels of 100g fasting, 1 hour, 2 hours, and 3 hours.

Table 1. Characteristics of research samples based on levels of 25(OH)D

| Variable n(%) | 25(OH)D Level | | | p |
|---|---|---|---|---|
| | Deficiency <20 ng/mL N = 35 | Insufficiency 20-29.9 ng/mL N = 15 | Normal > 30 ng/mL N = 12 | |
| Age (n,%) | | | | |
| 18-35 | 29(82.9) | 13(86.7) | 7(58.3) | 0.140 |
| > 35 | 6(17.1) | 2(13.3) | 5(41.7) | |
| Gestational age (n,%) | | | | |
| 24-34 weeks | 33(94.3) | 14(93.3) | 12(100.0) | 0.678 |
| >34 weeks | 2(5.7) | 1(6.7) | 0(0.0) | |
| Parity (n,%) | | | | |
| Primipara | 8(22.9) | 2(13.3) | 0(0.0) | 0.168 |
| Multipara | 27(77.1) | 13(86.7) | 12(100.0) | |
| Education level (n,%) | | | | |
| Low | 1(2.9) | 3(20.0) | 1(8.3) | 0.146 |
| Middle | 28(80.0) | 11(73.3) | 11(91.7) | |
| High | 6(17.1) | 1(6.7) | 0(0.0) | |
| Occupation (n,%) | | | | |
| Not Work | 26(74.3) | 11(73.3) | 10(83.3) | 0.793 |
| Work | 9(25.7) | 4(26.7) | 2(16.7) | |
| Sun exposure (n,%) | | | | |
| <90 minutes/day | 34(97.1) | 15(100.0) | 4(33.3) | 0.001 * |
| ≥90 minutes/day | 1(2.9) | 0(0.0) | 8(66.7) | |
| Food supply (n,%) | | | | |
| Never | 0(0.0) | 0(0.00) | 0(0.0) | 0.662 |
| Seldom | 33(94.3) | 13(86.7) | 11(91.7) | |
| Often | 2(5.7) | 2(13.3) | 1(8.3) | |
| Long sleeve clothes (n,%) | | | | |
| Yes | 33(94.3) | 14(93.3) | 2(16.7) | 0.001 * |
| No | 2(5.7) | 1(6.7) | 10(83.3) | |
| Use of sunscreen | | | | |
| Yes | 0(0.0) | 0(0.0) | 1(8.3) | 0.120 |
| No | 35(100.0) | 15(100.0) | 11(91.7) | |

* Statistically significant (p<0.05)

Table 2. Comparison of serum vitamin D between GDM and normal pregnant women (case n=31, control n=31)

| Variable n(%) | Group | | p |
|---|---|---|---|
| | GDM (Case) N = 31 | Control N = 31 | |
| 25(OH)D Level (n,%) | | | |
| Deficiency | 29 (93.5) | 6 (19.4) | 0.001* |
| Insufficiency | 2 (6.5) | 13 (41.9) | |
| Normal | 0 (0.0) | 12 (38.7) | |
| | | | |

* Statistically significant (p<0.05)

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for gestational diabetes (GDM) according to maternal plasma 25 -hydroxyvitamin D 25(OH)D concentrations in pregnancy

| 25(OH)D Level | Incident, n(%) | | OR | p | 95%CI | |
|---|---|---|---|---|---|---|
| | GDM N=31 | Control N=31 | | | Lower | Upper |
| Deficiency | 29(93.5) | 6(19.4) | 11.94 | 0.001* | 3.11 | 45.73 |
| Not Deficiency | 2(6.5) | 25(80.6) | | | | |

*Statistically significant (p < 0.05)

Table 4. Association of 25(OH)D levels with OGCT 50g

| Variable | OGCT 50g | p |
|---|---|---|
| | N=62 | |
| | Mean + SD | |
| 25(OH)D Level | | |
| Deficiency (<10 ng/mL) | 163.34 + 29.95 | |
| Insufficiency (20-29.9 ng/mL) | 139.67 + 33.69 | 0.001 [a*] |
| Normal(> 30 ng/mL) | 129.33 + 23.54 | |

*a. Non-parametric Mann-Whitney Test*

* Statistically significant (p<0.05)

**Discussion**

Total sample of this study were 62 pregnant women with gestational age > 24 weeks at Dr. Soetomo Hospital and Airlangga University Hospital Surabaya who met the inclusion and exclusion criteria. GDM was diagnosed according to the ADA guidelines, and it was established from the anamnesis, there was no increase in blood sugar levels when not pregnant and before 20 weeks of pregnancy. All samples of this study were treated equally. All research samples were asked and answered questions, physical examination and blood was taken to check levels of OGCT 50g, OGTT 100g fasting, 1 hour, 2 hours, 3 hours, and levels of 25(OH)D.

This study identified 10 characteristics of the study sample in relation to serum 25(OH)D levels with OGCT levels of 50g, OGTT 100g and the incidence of GDM. These characteristics were subject's age, gestational age, BMI, parity, mother's education level, occupation, sun exposure (minutes/day), food intake, long-sleeved clothing (covering arms and hands), and use of sunscreen. Of the overall research sample, the highest proportion of BMI is obesity as many as 26 samples, followed by overweight 25 samples, the rest are normal and underweight. Meanwhile, for exposure to sunlight < 90 minutes per day, there were more with 53 samples compared to exposure to sunlight > 90 minutes per day as many as 9 samples. For levels of 25(OH)D, the highest levels of deficiency were 35 samples, followed by insufficiency levels in 15 samples and normal levels in 12 samples.

In this study, overall, there were no significant differences in characteristics between samples with deficiency 25(OH)D, insufficiency and normal levels. However, on the characteristics of sun exposure and long-sleeved clothing, there was a significant relationship with $p=0.001$ ($p<0.05$). In this study, there was a significant relationship between sun exposure < 90 minutes per day in samples with 25(OH)D deficiency with $p = 0.001$. Where obtained 34 (97.1%) samples who get exposure to sunlight < 90 minutes per day in the deficiency group. This is also in line with the characteristics of the long-sleeved clothing that the sample wears when leaving the house. In the sample that when leaving the house wearing long-sleeved clothes, 33 (94.3%) samples have a 25(OH)D deficiency level with $p = 0.001$. These results are in line with those presented by Holick in 2009 who stated that the largest source of vitamin D in humans is sun exposure. So if our bodies are not exposed to sunlight, especially UVB, we can experience a 25(OH)D deficiency. UVB radiation with a wavelength of 290-320 nm penetrates exposed skin and converts 7-dehydrocholesterol in the skin into previtamin D3. Season, time of day, length of day, clouds, fog, skin covering by melanin and sunscreen are factors that influence UVB radiation exposure and vitamin D synthesis (Holick, 2009).

Skin pigmentation as well as the use of topical sunscreens can inhibit UVB penetration into the epidermis by reducing 99% of the skin's capacity to produce vitamin D3 (Holick, 2009). Sunscreens with an SPF of 8 or more are able to inhibit UVB exposure although in general people do not use it in the right amount, and do not repeat the use of sunscreen regularly (Wolpowitz, 2006). In this study, almost all samples never used sunscreen when leaving the house, there was only 1 sample in the group whose 25(OH)D levels were at normal levels (30-100 ng/ml) who used sunscreen. So it was found that there was no significant relationship between the use of sunscreen with levels of 25(OH)D with $p=0.120$. In this study, there were no significant differences regarding sample age, gestational age, BMI value, parity, education, occupation in the sample with GDM compared to the control. However, the BMI results were relatively the same as the mean value in GDM 29.28 (17.90-38.80) and control 29.09 (21.50-45.60) with a higher proportion found in overweight and followed by class I obesity so that there was no significant relationship between BMI and GDM incident. This is not in accordance with what was stated by Alzaim in 2013 which states that obese women who have vitamin D deficiency have a higher risk of developing GDM compared to women who are not obese with sufficient amounts of vitamin D (Marcinkevage and Narayan, 2011).

From the results of the study, it was found that the GDM (case) group had a deficiency of 29 (93.5%), insufficiency 2 (6.5%) and no normal 25(OH)D levels were found. Meanwhile, in the control group, the deficiency was 6(19.4%), the insufficiency was 13(41.9%) and the normal value was 12(38.7%). The average value of 25(OH)D levels in GDM was 14.71 + 4.09 ng/ml and in control 28.90 + 9.10 ng/ml. A significance value of p=0.001 (p<0.05) was obtained, so it can be concluded that there was a significant relationship between 25(OH)D levels and the incidence of GDM. The lower the 25(OH)D level, the higher the incidence of GDM. And if the data is grouped into levels of deficiency and non-deficiency associated with the incidence of GDM, and analyzed by chi-square crosstab, the value of OR = 11.94 with 95% CI (3.11-45.73). which can be interpreted as a deficiency of 25(OH)D 11.94 times that increases the risk of developing GDM. These results are in agreement with a study conducted by Soheilykhah et al in 2013 investigating serum 25 hydroxyvitamin D at 24-28 weeks gestation in 204 Iranian pregnant women. Based on the oral glucose tolerance test, 26% of this group had GDM and 19% had IGT. Vitamin D deficiency was found in 78% of the group. Separately divided participants were divided into three groups: 54 women with GDM, 39 women with impaired glucose tolerance (IGT), and 111 women with normal glucose tolerance tests. Women with GDM and IGT had median serum concentrations of 25 hydroxyvitamin D significantly lower than normal control subjects (24 and 17 nmol/L versus 32nmol/L, respectively) (Alzaim, 2013).

In research conducted by Zhang et al (2008), Tacoma (2013), Liu et al (2011) and Mahmood (2021), similar results were also obtained that vitamin D (25(OH)D) deficiency was evident in women who developed GDM was compared in controls, which is consistent with most of the cross-sectional and case-control studies discussed in which GDM patients were more likely to be vitamin D deficient or have lower serum 25-hydroxyvitamin D. In Zhang et al's study, there was a 2.66 x risk of developing GDM in subjects with vitamin D deficiency. Based on research conducted by Zhang, et al in 2015 showed a consistent relationship between vitamin D deficiency and an increased risk of GDM. The results of a meta-analysis of 20 studies including 9209 participants showed that women with vitamin D deficiency had a significantly increased risk for GDM. The role of vitamin D in GDM, first 1.25 dihydroxy vitamin D is the active form of vitamin D, regulates the level of circulating glucose by binding to the vitamin D receptor of pancreatic cells and modulating insulin secretion. Second, 1.25(OH)2D3 increases insulin sensitivity by stimulating insulin receptor expression and increasing insulin response to glucose transport. Furthermore, 1.25(OH)2D3 regulates the balance between extracellular and intracellular calcium fluxes in cells, which is important for mediating tissue-responsive insulin-responsive intracellular processes (Mitri et al., 2011). Vitamin D also downregulates inflammatory mediators such as cytokines and NFkB and the Fas/Fas-L pathway, to suppress apoptosis of pancreatic cells (Liao, 2018).

In this study, a non-parametric Mann-Whitney test was conducted to analyze the relationship between 25(OH)D levels and 50g OGCT levels and found that serum 25(OH)D levels correlated with 50g OGCT levels with (p=0.001). The mean value of OGCT level is 50g at deficiency level 163.34 + 29.95, insufficiency level 139.67 + 33.69, normal level 129.33 + 23.54. These results are similar to the results of a study from Farrant, et al in 2013 in that study, there was an association found between maternal vitamin D status and the risk of gestational diabetes mellitus. Observations from this study support the idea that GDM is associated with lower vitamin D status, or that vitamin D deficiency increases the risk of developing GDM. A significant association was found that, together with the group of mothers with vitamin D deficiency, there was an association between serum 25-hydroxyvitamin D and 30 min glucose concentration after oral glucose load or OGCT 50g.

In this study, a non-parametric Kruskal-Wallis test was found that serum 25(OH)D levels in samples with GDM were not significantly associated with OGTT levels of 100g fasting, 1 hour, 2 hours, and 3 hours. With a successive significance value of OGTT 100g fasting p=0.747, OGTT 100g 1 hour p=0.148, OGTT 100g 2 hours p=1,000, OGTT 100g 3 hours p=0.469. This result was caused by the even distribution of the results of the 100 g OGTT which increased during fasting, 1 hour, 2 hours and 3 hours. In the study of Liu et al., in 2011 it was found that low vitamin D status can affect blood glucose metabolism by affecting the initial phase of glucose-induced insulin secretion after oral glucose load. So that the most significant increase in blood sugar levels was at the beginning when given a 50g OGCT oral glucose load, this is in accordance with our study which found a significant relationship between 25(OH)D levels and 50g OGCT levels. While the relationship with OGTT levels of 100g fasting, 1 hour, 2 hours, 3 hours did not get a significant relationship.

## Conclusion

Serum 25(OH)D levels are associated with the incidence of gestational diabetes mellitus, deficiency of 25(OH)D increases the risk of developing GDM, serum 25(OH)D levels are associated with OGCT 50g levels, serum 25(OH)D levels are not associated with OGTT levels of 100g fasting, 1 hour, 2 hours, and 3 hours.

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