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Serum 25-hydroxyvitamin D levels in relation to lipids and clinical outcomes in pregnant women with gestational diabetes mellitus: an observational cohort study

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ABSTRACT

Objective The aim of the present study was to investigate whether 25-hydroxyvitamin D (25(OH)D) status at 24–28 weeks is associated with blood lipids and pregnancy outcomes in patients with gestational diabetes mellitus (GDM).

Design We performed an observational cohort study.

Setting The study was conducted in China.

Participants A total of 261 pregnant women diagnosed with GDM at 24–28 weeks of gestation in our hospital were included between June 2015 and December 2017. According to the levels of 25(OH)D, the women were divided into the G1 (<20 ng/mL) and G2 (≥20 ng/mL) groups. The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), TG/HDL-c and TC/HDL-c ratios were obtained from medical records. Pregnancy outcomes included gestational weeks of birth and delivery mode. Newborn information included birth weight and body length. Differences between groups were tested with adjusted multiple linear regression.

Results The serum levels of 25(OH)D (14.1±3.4 ng/mL vs 28.5±6.5 ng/mL, p<0.001), TC (5.3±0.9 vs 5.6±0.8, p=0.006), HDL-c (1.8±0.4 vs 1.9±0.4, p=0.046) and LDL-c (2.5±0.6 vs 2.7±0.7, p=0.015) in the G2 group were significantly higher than those in G1 group, while TG/HDL-c ratios (1.43±0.7 vs 1.26±0.7, p=0.035) were significantly higher in the G1 group. Moreover, we failed to find a significant difference in pregnancy outcomes of mothers and newborns among the two groups (p>0.05). In models adjusting for maternal age, parity, height, blood pressure, socioeconomic status, educational attainment, pre-pregnancy body mass index, season and gestational age, maternal 25(OH)D was associated with TG/HDL-c ratios (B=−0.016; 95% CI= −0.025 to −0.006).

Conclusion We found that there was no relationship between vitamin D and pregnancy/neonatal outcomes in our study. Maternal 25(OH)D at 24–28 weeks was inversely associated with TG/HDL-c ratios.

Strengths and limitations of this study

► To the best of our knowledge, this is the first study to evaluate the association between 25-hydroxyvitamin D (25(OH)D) concentrations and lipid profile during pregnancy in a Chinese gestational diabetes mellitus (GDM) population based on the grouping of vitamin D levels.

► In this study, we designed a questionnaire containing clinically relevant factors, including age, body mass index, parity and seasons for women at different educational attainment.

► Serum 25(OH)D was measured after women were diagnosed with GDM, which enabled the assessment of longitudinal relationships between 25(OH)D and pregnancy outcomes in the GDM population.

► Confounding factors were the main limitations of this retrospective observational design.

► The cohort of women with GDM may not represent the general population of women with GDM due to the location of subject recruitment and small sample size.

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to different degrees of abnormal glucose metabolism first occurring or first identified in pregnancy, and it is one of the common complications during pregnancy. The total prevalence of GDM in China is 14.8%.[1] Because of the adaptations to pregnancy, pregnant women will have some physiological changes in lipid metabolism.[2]

Vitamin D is a necessary fat-soluble vitamin for the human body; it is involved not only in regulating calcium and phosphorus metabolism, but also in improving insulin resistance, regulating insulin secretion and blood lipid metabolism.[3–5] Studies have shown that vitamin D deficiency may be associated with increased risk of GDM and dyslipidaemia.[6] The lower the concentration of vitamin D in the second trimester, the higher the serum triglyceride (TG) or total cholesterol (TC).[7] Vitamin D deficiency is prevalent in pregnant women, and it is reported that 47% of Asian

pregnant women have a severe deficiency of vitamin D.[1] Vitamin D deficiency is also common among pregnant women in China. The TG/high-density lipoprotein cholesterol (HDL-c) ratio may be a good marker to identify insulin-resistant Chinese individuals[4] and is positively associated with diabetes risk.[8]

At present, there is no consistent conclusion about the correlation between vitamin D and lipid indices. The aim of our research was to investigate whether 25-hydroxyvitamin D (25(OH)D) status at the second trimester was associated with blood lipids and pregnancy outcomes in patients with GDM.

MATERIALS AND METHODS
Participants
This trial recruited pregnant women from the affiliated Drum Tower Hospital of Nanjing University Medical College in Nanjing, China, between June 2015 and December 2017. The majority (75%–85%) of pregnant women living in this area (latitude of 32°N) attend the Drum Tower Hospital for antenatal care. Pregnant women who attended the Clinical Nutrition Department of the Drum Tower Hospital were eligible to participate in this trial based on the study criteria.

The inclusion criteria were as follows: (1) age 20–40 years; (2) a plan to visit regularly for prenatal examination and give birth in the Drum Tower Hospital; and (3) a diagnosis of GDM at 24–28 weeks. The exclusion criteria were as follow: (1) unknown data of last menstrual period; (2) twin or multiple pregnancy; (3) abortions ≥3; (4) received assisted reproductive technology; (5) medical or surgical diseases; (6) syphilis, Hepatitis B virus or HIV carrier; (7) a diagnosis of gestational complications (except GDM); (8) previous diagnosis of gestational diabetes (or diabetes); (9) the use of insulin therapy or hypoglycaemic drugs and (10) alcohol abuse, smoking and long-term medication.

We used a self-administered questionnaire and the prenatal maternity card of these participants to collect the basic data (age, height, weight, educational attainment, socioeconomic stats, etc). Of the eligible pregnant women, 261 women were enrolled in the final study.

Assessment of GDM and vitamin D deficiency
We included pregnant women who were subjected to a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks. According to the International Association of Diabetes and Pregnancy Study Groups Recommendations guidelines, the diagnosis of GDM was based on the 75 g OGTT. GDM was diagnosed if the OGTT results equalled or exceeded the following values: fasting plasma glucose ≥5.3 mmol/L, OGTT 1 hour ≥10.0 mmol/L and OGTT 2 hour ≥8.6 mmol/L.[9]

As a part of the general care of GDM pregnant woman, total serum 25(OH)D levels are routinely measured in the second trimester after were GDM diagnosis. According to the Endocrine Society Clinical Practice Guideline, vitamin D deficiency is defined as 25(OH)D of <20 ng/mL, insufficiency as 25(OH)D of 20–30 ng/mL and sufficiency as 25(OH)D of ≥30 ng/mL.[10] Pregnant women with serum 25(OH)D concentration <20 ng/mL were classified into the G1 group (n=113), and those with serum 25(OH)D concentration ≥20 ng/mL were classified into the G2 group (n=148).

Pregnancy outcomes and neonatal outcomes
The clinical outcomes of mothers included gestational weeks of birth and delivery mode. The neonatal outcomes, including birth weight (g) and body length (cm), were retrospectively collected from birth records.

Informed consent is waived by the ethics committee because of the retrospectively study design.

Statistical analysis
We compared lipid levels and pregnancy outcomes between the two groups (n=113 in the G1 group and n=148 in the G2 group). The normality of the variables was visually inspected. Continuous variables were compared using the Mann-Whitney U-test or Kruskal-Wallis H test, while categorical variables were compared using the χ^2 test. The measurement data were expressed as the mean±SD ($\bar{x}\pm s$), and skewed variables were expressed as median (25th percentile and 75th percentile). Moreover, Spearman's correlation analysis and multiple linear regression analysis were used to evaluate the relationship between 25(OH)D and blood lipids. The relevant confounding variables controlled for the multiple regression analysis included age, height, pre-pregnancy body mass index (BMI), parity, blood pressure, socioeconomic status, educational attainment and season of blood sampling, and 95% CIs were calculated. P<0.05 was considered statistically significant. Statistical analyses were performed using SPSS software V.22.0.

RESULTS
Of the eligible pregnant women, 441 patients who met the inclusion criteria were enrolled. The following patients were excluded: 92 patients did not complete the nutrition-related examinations; and 88 patients missed the normal time of antenatal care. In total, 261 women were included in the final population (online supplemental figure 1).

According to 25(OH)D levels, we divided the patients into G1 and G2 groups. In this study, the mean age of pregnant women with GDM was 30.1±4.0 years old, and the mean concentration of serum 25(OH)D was 22.2±9.0 ng/mL. Vitamin D deficiency in this population accounted for 43.1% (25(OH)D <20 ng/mL), and 17.6% women had sufficient concentrations of 25(OH)D (table 1).

The groups were generally comparable across the background variables, and there were no statistically significant differences in age (p=0.228), height (p=0.307), weight (p=0.919), pre-pregnancy BMI (p=0.588) and gravida status (p=0.682) between the two groups. There

Table 1 Characteristics of pregnant women according to 25(OH)D levels

Group	All		G1 <20 ng/mL		G2 ≥20 ng/mL		P*
n	261		113		148		
Age (years)	30 (27,33)		30 (27,32)		30 (27,33)		0.228
Height (cm)	161.3	4.9	161.5	4.6	161.2	5.1	0.307
Weight (kg)	59.8	10.6	59.0	10.3	60.4	10.8	0.919
Pre-pregnancy BMI (kg/m^2)	23.0	3.9	22.8	3.8	23.1	4.0	0.588
Blood pressure (mm Hg)							
Systolic blood pressure	110.4	5.7	110.1	6.3	110.6	5.3	0.518
Diastolic blood pressure	66.4	3.0	66.2	3.6	66.5	2.5	0.345
Monthly income (%)							0.530
<3000	1.9		3		1.4		
3000–6000	50.2		46.9		53.8		
>6000	47.9		50.4		46.9		
Education attainment (years) (%)							0.532
≥16	78.6		80.4		77.2		
9–12	19.5		17.0		21.4		
≤6	1.9		2.7		1.4		
Gravida status, n(%)							0.682
1	53.3		52.2		54.1		
2	29.5		30.1		29.1		
≥3	17.2		17.7		16.8		
Season of blood sampling (%)							0.069
Spring	22.2		27.4		18.2		
Summer	36.0		27.4		42.6		
Autumn	17.6		18.6		16.9		
Winter	24.1		26.5		22.3		

*P values refer to differences between the groups. Normally distributed variables were expressed as mean±SD. Skewed variables were expressed as median (25th percentile, 75th percentile).
BMI, body mass index.

was no significant difference in 25(OH)D levels between women with different educational attainment (p=0.532) and socioeconomic status (p=0.530). Based on the season of blood sampling, we divided the seasons into spring (from March to May), summer (from June to August), autumn (from September to November) and winter (from December to February). We observed that the prevalence of maternal vitamin D deficiency (25(OH)D <20 ng/mL) was higher in winter and lower in summer (table 1).

The results showed that mothers with higher levels of vitamin D had higher TC (p=0.006), HDL-c (p=0.046) and low-density lipoprotein cholesterol (LDL-c) (p=0.015). The TG/HDL-c ratio was significantly higher in the 25(OH)D deficiency group (p=0.035). No statistically significant difference was observed between the two groups in terms of TG and TC/HDL-c (table 2).

Regarding the pregnancy outcomes and newborn data, differences between the groups were not significant (p>0.05; table 3).

Spearman's correlation analysis between vitamin D and lipid indices showed that the concentration of 25(OH)D in GDM patients was negatively correlated with TG (r=−0.146, p=0.018) and TG/HDL-c (r=−0.185, p=0.003) but positively correlated with TC (r=0.150, p=0.016) and HDL-c (r=0.197, p=0.001).

Separate linear regression analysis revealed that 25(OH)D had a positive correlation with TC, HDL-c and TG/HDL-c ratios (p<0.05 for all). After adjusting for maternal age and pre-pregnancy BMI, 25(OH)D had a positive correlation with HDL-c (p=0.046; model 1, table 4). However, after including socio-economic status in the models, maternal 25(OH)D was no longer associated with HDL-c. In models with additional adjustment for parity, educational attainment, height, blood pressure, socioeconomic status, season and gestational age, maternal 25(OH)D was inversely associated with TG/HDL-c ratios (B=−0.016; 95% CI = −0.025 to 0.006; model 2, table 4). Although the model of TG/HDL-c

Table 2 Lipid profile indicators and vitamin D levels in the same period

Group	All		G1 <20 ng/mL		G2 ≥20 ng/mL		P*
25(OH)D (ng/mL)	22.2	9.0	14.1	3.4	28.5	6.5	0.000
TG (mmol/L)	2.3	0.8	2.4	0.9	2.2	0.8	0.101
TC (mmol/L)	5.4	0.9	5.3	0.9	5.6	0.8	0.006
HDL-c (mmol/L)	1.9	0.4	1.8	0.4	1.9	0.4	0.046
LDL-c (mmol/L)	2.6	0.7	2.5	0.6	2.7	0.7	0.015
TG/HDL-c	1.33	0.7	1.43	0.7	1.26	0.7	0.035
TC/HDL-c	2.99	0.7	3.01	0.7	2.98	0.7	0.979

Normally distributed variables were expressed as mean±SD.
*P values refer to differences between the groups.
HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TC, total cholesterol; TG, triglyceride.

and 25(OH)D was statistically significant (p=0.036), the model was not sufficiently interpreted (R^2=6.9%).

DISCUSSION

In this study, we found a high incidence of vitamin D deficiency in pregnant women with GDM. After adjusting for age, pre-pregnancy BMI, parity, height, blood pressure, socioeconomic status, educational attainment, season and gestational age, maternal 25(OH)D at 24–28 weeks was inversely associated with TG/HDL-c ratios. Moreover, there was no relationship between vitamin D and pregnancy outcomes, newborn weight and newborn length.

In the present study, maternal 25(OH)D in GDM patients was negatively correlated with triglycerides (r=−0.146) and TG/HDL-c (r=−0.185) while positively correlated with TC (r=0.150) and HDL-c (r=0.197), which was consistent with previously reported results for maternal 25(OH)D and blood lipid-related indicators.[11] However, the present results contradicted the results reported by Lepsch, who studied the relationship research between vitamin D and TC.[12] This difference may be related to the selection of GDM population. In addition, pregnant women have a certain degree of physiological increase of TC. There were no statistically significant differences in gestational weeks and caesarean section rate between the two groups, which disagreed with the research reported by Merewood et al.[13] There was no independent relationship between

vitamin D concentration and newborn outcomes, which was consistent with the research results of a multi-ethnic population-based study[14] and a meta-analysis.[15] Vitamin D levels have been shown to correlate with blood glucose and lipid index in the first trimester.[9] In our city, early pregnancy tests are generally performed at community hospitals. Considering that the community measurement methods are not unified and data collection is incomplete, we did not conduct the relevant research. Thus, we were unable to verify the changes of the pregnancy.

During the entire pregnancy, the physiological state and metabolism of pregnant women is greatly changed, especially the endocrine system and insulin secretion. Increased levels of insulin in the circulatory system lead to abnormal glucose tolerance of pregnant women and an increased incidence of gestational diabetes. In recent years, several studies have found that vitamin D acts as a hormone. Studies have shown that low vitamin D concentration affects glycaemic control in patients with gestational diabetes.[8 16] Vitamin D is a steroid derivative that is mainly stored in adipose tissue. Investigation of the effect of vitamin D intervention on glucose and lipid metabolism in patients with type 2 diabetes[17] has shown that vitamin D affects glucose and lipid metabolism in patients.

In patients with diabetes and insulin resistance, dyslipidaemia is characterised by increased TG levels and decreased HDL-c levels.[18] Studies have shown that higher

Table 3 Pregnancy outcomes and newborn data

Group	All		G1 <20 ng/mL		G2 ≥20 ng/mL		P*
Gestational age at birth (weeks)	39 (38, 40)		39 (38, 40)		39 (38, 40)		0.733
Caesarean section (%)	33.0		31.9		33.8		0.845
Birth weight (g)	3390.6	461.2	3401.4	467.8	3382.4	457.5	0.694
Crown–heel length (cm)	50.2	2.0	50.3	1.7	50.1	2.2	0.412

Normally distributed variables were expressed as mean±SD, skewed variables were expressed as median. (25th percentile, 75th percentile).
*P values refer to differences between the groups.

Table 4 Multiple linear regression analysis of the association between serum 25(OH)D levels with other factors

Outcomes	Univariate analysis				Multiple analysis (model 1)				Multiple analysis (model 2)			
	B	(95% CI)	P*	R²	B	(95% CI)	P*	R²	B	(95% CI)	P*	R²
TG (mmol/L) 25(OH)D	−0.011	−0.023 to 0.000	0.059	0.014	−0.011	−0.023 to 0.001	0.062	0.015	−0.015	−0.027 to −0.003	0.147	0.052
TC (mmol/L) 25(OH)D	0.015	0.004 to 0.027	0.009	0.027	0.015	0.004 to 0.027	0.052	0.030	0.014	0.002 to 0.026	0.280	0.043
HDL−c (mmol/L) 25(OH)D	0.009	0.003 to 0.014	0.002	0.037	0.009	0.003 to 0.014	0.046	0.047	0.010	0.004 to 0.016	0.085	0.059
LDL−c (mmol/L) 25(OH)D	0.006	−0.003 to 0.015	0.158	0.008	0.006	−0.003 to 0.015	0.566	0.008	0.008	−0.002 to 0.017	0.763	0.023
TG/HDL−c 25(OH)D	−0.013	−0.022 to −0.004	0.006	0.025	−0.013	−0.022 to −0.004	0.056	0.018	−0.016	−0.025 to −0.006	0.036	0.069

*P values refer to differences between the groups.

HDL−c, high-density lipoprotein cholesterol; LDL−c, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TC, total cholesterol; ; TG, triglyceride.

serum TG or TC levels in the second trimester result in lower patient's 25(OH)D concentrations, which may lead to the higher risk of GDM.[7 19 20] This phenomenon may provide the basis for early prediction, diagnosis and treatment of GDM, thereby reducing the risk of adverse pregnancy outcomes.[21] The TG/HDL-c ratio may be a good marker to identify insulin-resistant Chinese individuals[4] and is positively associated with diabetes risk.[22 23] Our finding about TG/HDL-c ratios implied that it may be possible to decrease the TG/HDL-c ratio by increasing the concentration of 25(OH)D, which would be helpful for improving insulin resistance. In healthy normal pregnancies, we have previously observed that inadequate 25(OH)D concentrations during early pregnancy are associated with more pronounced changes of TC, LDL-c concentrations and TC/HDL-c ratios throughout pregnancy.[12] Previous study results are contradictive and do not clearly support therapeutic supplementation as a safe strategy for the prevention of pregnancy complications.[23] However, considering there was a high prevalence of vitamin D insufficiency in our study, we recommend that all pregnant women, especially those with GDM, be regularly tested and prompt treatment for women who are vitamin D deficient.

To the best of our knowledge, this is the first study to evaluate the association between 25(OH)D concentrations at 24–28 weeks and lipid profile during pregnancy in a GDM group in the Chinese population. In this study, we designed a questionnaire containing clinically relevant factors, including age, BMI, parity and seasons, for women at different educational attainment, and we trained students to help the pregnant women fill out the questionnaire. Serum 25(OH)D level was measured after women were diagnosed with GDM, which enabled assessment of longitudinal relationships between 25(OH)D and pregnancy outcomes in the GDM population. There were several limitations in this study. First, the sample size of this study was insufficient. We did not include healthy normal pregnant women due to the small sample size of healthy pregnant women participating in regular vitamin D checks, which had a certain impact on the results and could not explain the relationship between vitamin D levels and the incidence of GDM.[24] Second, our research measured total vitamin D. Measurements of free vitamin D might be more appropriate due to the stimulation of vitamin D-binding protein during pregnancy. Tsuprykov *et al* found that free 25(OH)D revealed better associations with markers of lipid metabolism.[25] Free vitamin D measurements are independent of confounding factors, such as pregnancy and genetic background, and may be much better correlated with the lipids and clinical outcome.[26] Third, in the process of data collection, the time that some pregnant women had serum 25(OH)D levels measured was not consistent with the biochemical examination time (differences up to 2 weeks), which may have led to numerical differences, thereby affecting the interpretation of the model.

In summary, our study found that HDL-c levels and TG/HDL-c ratios in patients with gestational diabetes during

the second trimester may be affected by serum 25(OH) D levels. It is necessary to perform regular testing and prompt treatment for women with GDM who are vitamin D deficient. The present research provided a foundation for future studies that aim to investigate the benefits of controlling blood lipids in pregnant women with GDM. Thus, further observational studies and interventional randomised controlled trials are still needed to confirm the association between blood lipids and 25(OH)D of GDM patients.

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Supplementary Figure 1   The flow diagram of the research

Assessed for eligibility (n=441)

Excluded (n= 92)
- Not meeting inclusion criteria (n=68)
- Declined to participate (n=5)
- Lack of complete medical records (n=9)
- Other reasons (n= 10)

Lost to follow-up (missed the normal time of antenatal care) (n= 88)

Analysed (n= 261)

G1 group: serum 25(OH)D ＜20ng/ml (n= 113)

G2 group: serum 25(OH)D ≥ 20ng/ml (n= 148)