Correlation of serum 25-hydroxyvitamin-D_3, hs-CRP, and homocysteine in women with gestational diabetes mellitus

Li Zhang, Huani Yi, Jie Li, Juan Dong

Department of Obstetrics and Gynecology, Jinan Maternity and Child Care Hospital, Jinan, China

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Corresponding author:
Juan Dong
Department of Obstetrics and Gynecology
Jinan Maternity and Child Care Hospital
Jinan, Shandong
China
e-mail: dongjuan0123@126.com

Abstract

Introduction: To explore the changes and correlation of serum 25-hydroxyvitamin-D3, high-sensitivity C-reactive protein (hs-CRP), and homocysteine (Hcy) in patients with gestational diabetes mellitus (GDM).

Material and methods: A total of 110 GDM patients from September 2019 to September 2020 constituted the GDM group, and 100 pregnant women with a normal 75 g oral glucose tolerance test (OGTT) throughout the same duration constituted the control group. General information of the patients was collected: maternal age, BMI, gravidity, parity, and the gestational week at the time of glucose tolerance screening. Serum levels of 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy were compared between the two groups. Pearson correlation analysis was used to evaluate the correlation between 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy, and homeostasis model assessment of insulin resistance index (HOMA-IRI) in GDM patients.

Results: Serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group and control group were 15.67 ±3.62 and 18.66 ±3.41 ng/ml, hs-CRP levels were 6.07 ±1.45 and 3.12 ±1.07 mg/l, and serum Hcy levels were 17.03 ±4.10 and 8.63 ±2.27 µmol/l. Serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group were substantially lower than those in the control group, while hs-CRP, Hcy, and HOMA-IRI in the GDM group were significantly higher than those in the control group ($p < 0.05$). Pearson correlation analysis demonstrated that serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group were negatively correlated with HOMA-IRI. In contrast, hs-CRP and Hcy levels were substantially positively correlated with HOMA-IRI ($p < 0.01$).

Conclusions: Blood levels of 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy levels can be a potential indicator of GDM.

Key words: gestational diabetes mellitus, 25-hydroxyvitamin-D_3, high-sensitivity C-reactive protein, homocysteine.

Introduction

Pregnant women are mostly diagnosed with gestational diabetes mellitus (GDM) during the glucose tolerance screening at 24 to 28 weeks of gestation. The cause of the disease is currently unknown and may be related to genetic factors, early pregnancy diet, and placental insulin resistance [1, 2]. Studies [3, 4] have shown that the pathogenesis of GDM is closely associated with insulin resistance (IR), and it is considered one of the main pathogeneses of GDM. The latest research [5] revealed that some patients with type 2 diabetes have low vitamin D levels. Vitamin D

deficiency can affect insulin sensitivity by affecting the incidence of type 2 diabetes through immunological mechanisms. 25-hydroxyvitamin-D_3 (25-$(OH)_2$-$VitD_3$) is related to the decrease in overall insulin secretion and can be used as an indicator for early assessment of the vitamin D status of pregnant women [6–8]. Additionally, studies have demonstrated that inflammatory factors such as serum hypersensitive C-reactive protein (hs-CRP) and homocysteine (Hcy) are associated with the occurrence and development of type 2 diabetes [9–11]. This study aims to compare the differences in serum 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy levels between pregnant women with GDM and pregnant women with normal glucose tolerance, explore the correlation between the levels of 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy and the pathogenesis of GDM, and clarify the specific roles of 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy in GDM, providing new ideas for GDM diagnosis and treatment.

Material and methods

General information

One hundred ten pregnant women with GDM from September 2019 to September 2020 comprised the GDM group, and 100 pregnant women with a normal 75 g oral glucose tolerance test (OGTT). throughout the same duration comprised the control group. General information of the patients, such as the age of pregnant women, gestational weeks at the time of glucose tolerance screening, body mass index (BMI) value, gravidity, and parity, was collected. The ethics committee of Jinan Maternity and Child Care Hospital approved this study.

Inclusion criteria

GDM group: (1) comply with the definition of GDM in the Guidelines for the Diagnosis and Treatment of Diabetes Mellitus in Pregnancy (2014) [12]: patients met 2 of the following indicators in the results of the 75 g OGTT at 24 to 28 weeks of pregnancy: fasting plasma glucose (FPG) ≥ 5.6 mmol/l, 1 h postprandial plasma glucose (1 hPG) ≥ 10.3 mmol/l, 2 hPG ≥ 8.6 mmol/l and 3 hPG ≥ 6.7 mmol/l; (2) GDM was diagnosed for the first time without targeted intervention; (3) cognitive function was normal; (4) signed the informed consent.

Control group: (1) all inspection results were within the normal range; (2) no history of GDM; (3) singleton pregnancy; (4) signed the informed consent.

Exclusion criteria

Exclusion criteria: (1) patients with severe pregnancy complications such as hypertension during pregnancy; (2) patients with systemic infectious diseases; (3) patients with liver and kidney diseases that can cause fundamental lipid metabolism abnormalities; (4) patients with severe gastrointestinal dysfunction, leading to abnormal metabolism of basic vitamin D_3.

Indicators and methods of detection

The levels of serum 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy, and homeostasis model assessment of insulin resistance index (HOMA-IRI) were compared between two groups, and the correlations between serum 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy, and HOMA-IRI in GDM patients were analyzed.

Three milliliters of cubital venous blood was collected after fasting for 8 h in both groups. Centrifuged after standing, the upper serum samples were collected for testing, using the ELISA method (American Radu RT-6000 enzyme label instrument) to determine the serum 25-$(OH)_2$-$VitD_3$ level, and the relevant procedures were carried out in strict accordance with the instructions of the kit (Shanghai Hengyuan Biotechnology Co., Ltd.). Serum hs-CRP and Hcy levels were determined by fluorescence polarization immunoassay and immunoturbidimetry respectively, and the procedures were performed in strict accordance with the kit instructions (Shanghai Hengyuan Biotechnology Co., Ltd.). The fasting insulin (FINS) level was measured using the Beckman Coulter (China) UniCelDxI800 automatic chemiluminescence immunoassay analyzer, and the HOMA-IRI was calculated by the formula HOMA-IRI = FINS × FPG/22.5.

Statistical analysis

The SPSS Statistics package (version 26.0; IBM Corp., Armonk, NY) was used for all statistical analyses. All continuous variables data following data normal distribution were shown as the mean ± standard deviation. Statistical differences between the two sample means were assessed by the *t*-test for continuous variables, and the correlation analysis was performed by Person correlation analysis. All continuous variables' data not following a normal distribution are expressed as median, 25th percentile, and 75th percentile. The Mann-Whitney *U* test was used for comparison between two groups, and the correlation analysis were assessed by Spearman correlation analysis. A value of $p < 0.05$ was considered statistically significant.

Results

General clinical data

Overall 210 patients were included in this study, comprising 110 cases in the GDM group

and 100 cases in the control group. There were no significant differences between the two groups in maternal age, BMI, gravidity, parity, and the gestational weeks at the time of glucose tolerance screening ($p > 0.05$). The general clinical information of the patients is provided in Table I.

Comparison of 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy levels, and HOMA-IRI

Serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group and control group were 15.67 ±3.62 ng/ml and 18.66 ±3.41 ng/ml, hs-CRP levels were 6.07 ±1.45 and 3.12 ±1.07 mg/l, and serum Hcy levels were 17.03 ±4.10 and 8.63 ±2.27 µmol/l. There were 42 cases of vitamin D deficiency in the GDM group, and the incidence of vitamin D insufficiency was 38.18% (42/110). There were 14 cases of vitamin D deficiency in the control group, and the incidence of vitamin D insufficiency was 14% (14/100). Between the two groups of subjects, serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group were significantly lower than those in the control group. In contrast, hs-CRP, Hcy, and HOMA-IRI in the GDM group were substantially higher than those in the control group ($p < 0.05$) (Table II).

Correlation analysis of 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy levels, and HOMA-IRI

Pearson correlation analysis revealed that serum 25-$(OH)_2$-$VitD_3$ levels in the GDM group were negatively correlated with HOMA-IRI ($r = –0.717$, $p < 0.05$) (Figure 1), and hs-CRP levels were strongly positively correlated with HOMA-IRI ($r = 0.781$, $p < 0.01$) (Figure 2). There was a strong positive correlation between Hcy levels and HOMA-IRI ($r = 0.790$, $p < 0.01$) (Figure 3).

Discussion

At present, the pathogenesis of GDM is still unclear [13]. High insulin levels during pregnancy are one of the signs of IR. Changes in secretion and

Table I. Comparison of general clinical data between the two groups (mean ± SD, *n*)

Group	Cases	Age	Gestational weeks at the time of glucose tolerance screening	BMI (kg/m²)	Gravidity	Parity
GDM group	110	29.74 ±5.19	25.19 ±2.05	25.71 ±3.84	2.54 ±0.48	0.73 ±0.12
Control group	100	29.84 ±6.07	25.28 ±2.17	25.69 ±3.74	2.61 ±0.45	0.75 ±0.18
Value of *p*		0.897	0.758	0.969	0.278	0.341

BMI – body mass index, GDM – gestational diabetes mellitus.

Table II. Comparison of serum 25-$(OH)_2$-$VitD_3$, hs-CRP, Hcy levels, and HOMA-IRI between the two groups (mean ± SD)

Group	Cases	25-$(OH)_2$-$VitD_3$ (ng/ml)	hs-CRP (mg/l)	Hcy (µmol/l)	HOMA-IRI
GDM group	110	15.67 ±3.62	6.07 ±1.45	17.03 ±4.10	3.67 ±1.12
Control group	100	18.66 ±3.41	3.12 ±1.07	8.63 ±2.27	2.47 ±1.07
Value of *t*		6.142	–19.968	–18.570	–7.963
Value of *p*		< 0.01	< 0.01	< 0.01	< 0.05

hs-CRP – high-sensitivity C-reactive protein, Hcy – homocysteine, HOMA-IRI – homeostasis model assessment of insulin resistance index, GDM – gestational diabetes mellitus.

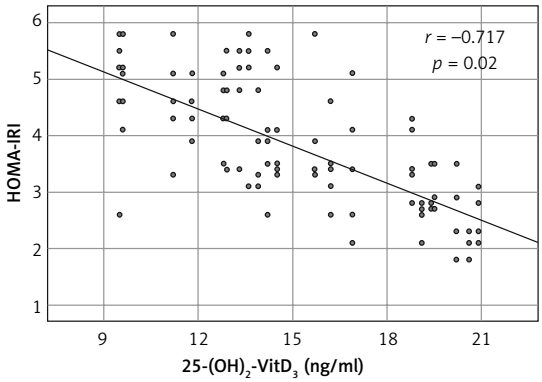

Figure 1. Scatter diagram of 25-$(OH)_2$-$VitD_3$ and HOMA-IRI

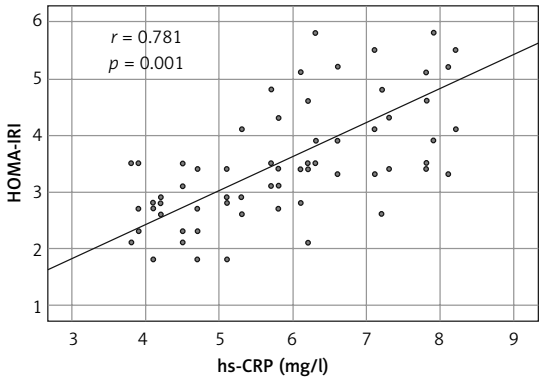

Figure 2. Scatter diagram of hs-CRP and HOMA-IRI

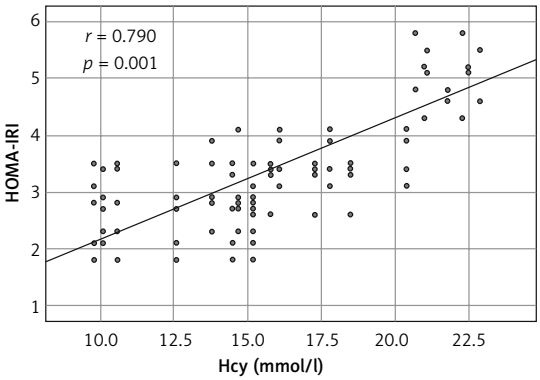

Figure 3. Scatter diagram of Hcy and HOMA-IRI

metabolism of GDM patients during pregnancy are among the most critical causes of IR [14]. During pregnancy, a large number of glucose-increasing hormones are produced, such as pituitary hormones, glucagon, placental lactogen, and steroid hormones. With the continuous progress of the pregnancy, blood volume increases, and blood dilution occurs, resulting in insufficient insulin [4]. To maintain a normal state of glucose metabolism, the secretion of insulin will continue to increase. Once this physiological compensatory mechanism is unbalanced and cannot regulate blood glucose, it will cause blood glucose to rise, resulting in GDM [15]. In this study we found that the HOMA-IRI of the GDM group was higher than that of the control group, suggesting IR in GDM patients.

Vitamin D is a steroid hormone, which is well known because of its calcium and phosphorus regulating effect [16]. Vitamin D is a fat-soluble vitamin, which can be obtained from food. It is synthesized by 7-dehydrocholesterol in human skin under ultraviolet radiation. It forms a series of metabolites through the hydroxylation of the liver and kidney, which can regulate calcium and phosphorus metabolism and promote bone calcification, with autoimmune properties such as lowering insulin levels, anti-inflammatory, and immune regulation, affecting the human immune, blood, and endocrine system [17, 18]. In recent years, studies [19, 20] have demonstrated that it is also involved in insulin synthesis, secretion, and peripheral effects, so the function of low vitamin D status in the pathogenesis of GDM has received more and more attention. In this study, 25-$(OH)_2$-$VitD_3$, as the active ingredient of vitamin D in the serum of the GDM group, was substantially lower than that of the control group at the same pregnancy stage ($p < 0.05$) and negatively correlated with HOMA-IRI, suggesting that 25-(OH)2-$VitD_3$ deficiency is involved in the occurrence of GDM. The mechanism may be related to IR.

In recent years, studies [21] have revealed that GDM is closely related to inflammation, and low-level systemic chronic inflammation is one of the pathogeneses of GDM. Alamolhoda *et al.* [22] reported that inflammation can be associated with increased risk of GDM, and inflammatory factors such as CRP, IL, adiponectin, and TNF-α can affect the secretion of insulin by pancreatic islet B cells to increase blood glucose, leading to the occurrence of GDM [23]. Hyperglycemia caused by IR during pregnancy can stimulate the inflammatory cells in the body to release inflammatory factors. A variety of inflammatory factors play an essential function in the initiation and development of GDM [24]. hs-CRP is one of the most sensitive indicators of inflammation. When inflammation, trauma, and other diseases occur in the body, hs-CRP can increase significantly, which can be used as a potential indicator for GDM [25, 26]. Qiu *et al.* [27] conducted a prospective study on the relationship between serum hs-CRP and GDM in early pregnancy, and found that the relative risk of developing GDM in patients with elevated serum hs-CRP levels was 3.5 times higher than that of the control group. For every 1 mg/L increase in serum CRP, the risk of GDM increases by 20%. Hence, an increased hs-CRP level is one of the high-risk pathogenic factors of GDM and can be used as an indicator for early diagnosis of GDM. In our study we found that the serum hs-CRP levels of the GDM group were higher than those of the control group, suggesting that the serum hs-CRP levels of GDM patients were elevated. The serum hs-CRP levels were positively correlated with HOMA-IRI, implying that the higher the serum hs-CRP levels are, the higher is the HOMA-IRI. The levels of hs-CRP are related to the severity of GDM, and the levels of hs-CRP can indicate the occurrence and development of GDM.

Homocysteine (Hcy), as an intermediate product in methionine metabolism, has apparent vascular damage effects [28]. Increased Hcy concentration can induce oxidative stress through self-oxidation, which damages the function of vascular endothelial cells. It can cause microcirculation disorders by causing the aggregation and precipitation of lipoproteins, promoting platelet aggregation and thrombosis, participating in the pathophysiological process of decreased insulin secretion, and insulin resistance in diabetic patients [29, 30]. Vermeulen *et al.* [31] reported that Hcy levels were significantly increased in patients with GDM, which were near related to decreasing insulin sensitivity. In this study, the results showed that serum Hcy and HOMA-IR in the GDM group were substantially higher than those in the control group ($p < 0.05$). Correlation analysis showed a significant positive correlation between Hcy and HOMA-IRI ($p < 0.05$), indicating that elevated Hcy levels may cause IR, which is significant in the pathogenesis and progression of GDM.

In conclusion, the serum 25-$(OH)_2$-$VitD_3$ levels of GDM patients were significantly decreased, and

CRP and Hcy levels were significantly increased, which may be relevant in the occurrence of GDM. Blood levels of 25-$(OH)_2$-$VitD_3$, hs-CRP, and Hcy levels can be a potential indicator of GDM. Also, it is clinically possible to prevent and treat GDM through appropriate supplementation of vitamin D. Proper vitamin D supplementation can be used to prevent and treat GDM.

Conflict of interest

The authors declare no conflict of interest.

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