The vitamin D status is negatively correlated to glucose metabolism in Chinese patients with gestational diabetes

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Abstract

Background: Vitamin D deficiency plays a role in the development of patients with gestational diabetes (GDM). However, the effects and mechanism of vitamin D on fasting blood glucose are conflicting. This research aimed to explore the status of vitamin D in patients with GDM, its effect on fasting blood glucose and HbA1c, and to clarify the possible mechanism of vitamin D on glucose metabolism of patients with GDM.

Methods: From January 1, 2017, to March 31, 2018, we included all the hospitalized patients with GDM. The main indices were fasting blood glucose (FBG) and 25-hydroxy vitamin D [25(OH) D], glycosylated hemoglobin (HbA1c), fasting insulin (FINS), fasting peptide (FPC), parathyroid hormone (iPTH), serum creatinine (SCr), blood neutrophil count, lymphocyte count, blood calcium (Ca), and blood phosphorus (P), neutrophil count (Ne), lymphocyte count (Lym), neutrophil-to-lymphocyte ratio (NLR). *Results* The mean 25(OH) D concentration of 33 patients with GDM was 21.40±10.68 ng/mL, among them, the patients with vitamin D insufficiency was 30.30% (10 cases), the patients with vitamin D deficiency was 60.61%(20 cases), the patients with normal vitamin D level was 9.1%(3 cases). Adjusted by age, gestational age, and glomerular filtration rate (eGFR), 25(OH)D concentration were negatively correlated to and FBG (β = -0.587, p =0.001), HbA1c (β = -0.408, p =0.018), respectively. There was a negative correlation between 25(OH)D concentrations and NLR (β = -0.389, p = 0.035), adjusted by eGFR, gestational age, and age.

Conclusions: The status of vitamin D deficiency in patients with GDM is common. With the decrease of 25(OH) D concentration, fasting blood glucose and HbA1c increase. 25(OH) D concentration is negatively correlated to NLR. Vitamin D may affect fasting blood glucose and HbA1c in patients with gestational diabetes by regulating inflammation.

Background

The prevalence of vitamin D insufficiency or deficiency is high in women with pregnancy[1]. Homles et al. [2] found that both pregnant and nonpregnant women have vitamin D deficiency. Li found the pregnant women with vitamin D deficiency (< 75 nmol/L) was 62.34%, and the proportion of severe deficiency (< 25 nmol/L) was 0.25%[3]. Vitamin D deficiency, defined as serum 25(OH) D concentration above 50 nmol/L, is found to be common globally in pregnancy with a prevalence of 26 to–98%[4]. Some researches in the Chinese population have found that the status of vitamin D in patients with GDM is lower than that in pregnant women with normal blood glucose[5]. GDM has been recognized as a risk factor for many complications of pregnant women and fetus. In the short term, it increases the risk of C-section[6] [7], gestational hypertensive disorders [8], preterm birth [9], and macrosomia [6]. In the long term, it also increases the risk of type 2 diabetes and obesity for both mothers and the offspring in the future [10, 11].

Vitamin D deficiency may affect islet function and blood glucose control in patients with type 2 diabetes. Little information is found about the effect of vitamin D on blood glucose of patients with GDM. Meabh Walsh suggested in the first trimester of pregnancy, 25OHD was negatively associated with blood glucose, avoiding maternal vitamin D deficiency in early pregnancy is associated with lower blood glucose in early pregnancy and throughout pregnancy[6]. However, the relationship between vitamin D and blood glucose in patients with GDM is conflicting. Naseh found there is no relationship between maternal serum vitamin D levels and maternal or neonatal serum glucose or insulin levels [6]. Maryam Akbari did not found any beneficial effect of vitamin D supplementation on fasting plasma glucose (FPG), insulin, HbA1c[7]. Moreover, the effect and mechanism of vitamin D on the islet function in gestational diabetes is not clear. Research[5] showed that the neutrophil lymphocyte ratio (NLR) was significantly associated with GDM occurrence in patients with GDM, NLR could suggest GDM development and inflammatory response. Many research suggested vitamin D could ameliorate the inflammation. The pathogenesis of GDM is mainly related to insulin resistance during pregnancy, such as relative reduction of insulin secretion, tissue sensitivity to insulin, and autoimmune-mediated inflammation of islet cells [8]. Base on the researches above, we hypothesis that vitamin D may affect fasting blood glucose or HbA1c in patients with GDM via inflammation response. This study aimed to explore the status of vitamin D in patients with GDM and whether it may affect fasting blood glucose and or HbA1c in patients with GDM via inflammation response.

Subjects And Methods

2.1 Materials and Methods.

We identified 33 patients with GDM who were treated in the inpatient Department of Endocrinology and Metabolism in the Second Hospital affiliated Xiamen medical college from January 2017 to March 2018. According to the GDM diagnostic criteria of the American Diabetes Association (ADA) in 2011, the normal values of fasting, 1-hour, 2-hour venous blood glucose were, respectively, 5.1 mmo/L□10.0mmoL/L□ 8.5 mmol/L, any blood glucose value greater than the normal value can be diagnosed as GDM. According to Endocrine Society Clinical Practice Guidelines on vitamin D deficiency, serum circulating 25-hydroxyvitamin D [25(OH)D] concentrations were measured to evaluate vitamin D status[9]. Five of them refused to test the concentration of 25(OH) D. A total of 33 subjects were finally enrolled. The following criteria were used to include subjects: 1. confirmed GDM, 2.serum iPTH at 15.0–65.0 pg/mL, 3.blood calcium less than 2.45 mmol/L, 4. normal liver function, serum creatinine, urea nitrogen, electrolytes, 5.without medication of insulin, insulin analogs, vitamin D and vitamin D analogs. The following criteria were used to exclude subjects:1.Women with a history of diabetes, 2.metabolic bone diseases,3.liver dysfunction or impaired kidney function 4.non-pregnant diabetes 5.patients with diabetic ketoacidosis, diabetic ketosis, diabetic hyperosmotic state, 6.blood phosphorus > 1.60 mmol/L, 7. acute infection, 8. tumor, 9. refused to participate in the group.

2.2 Groups

Vitamin D deficiency was defined as 25(OH)D of ≤ 20 ng/mL [≤ 50 nmol/L], vitamin D insufficiency was defined as a 25(OH)D between 20 ng/mL [50 nmol/L] and 30 ng/mL[75 nmol/L]. The normal serum 25(OH)D concentration was defined as ≥ 30 ng/mL [≥ 75 nmol/L], according to the Endocrine Society Clinical Practice Guideline of vitamin D deficiency[9]. The subjects were divided into three groups according to serum 25(OH)D value: (G1) vitamin D deficiency [25(OH)D ≤ 20 ng/mL (50 nmol/L)], (G2) vitamin D insufficiency [25(OH)D value between 20 ng/mL (50 nmol/L) and 30 ng/mL (75 nmol/L)],and (G3) normal vitamin D status [25(OH)D ≥ 30 ng/mL (75 nmol/L)].

2.3 Anthropometric and Biochemical Analysis

(1) Data were gathered on age, sex. Participants' weights and heights were recorded. As all the patients were pregnant, bodymass index (BMI) was not calculated. The body weights and heights were measured while the subjects wore light clothing without shoes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured while sitting, after a 5-minute rest, and again after a10 minute intervals, and the mean values were recorded. Hypertension was also diagnosed with a history of antihypertensive medication.

Blood samples were drawn between 08:00 am and 09:00 am for laboratory analysis of biochemical variables [25(OH)D, HbA1c, FBG, FINS, FPC, Ca, P]. We follow the method of anthropometric and biochemical analysis in another research we published before[10]. FINS [insulin kits, Roche Diagnostics (Shanghai); Roche Cobas 6000 analyzer], C-peptide (C-peptide kits, Roche Diagnostics; Roche Cobas 6000 analyzer), and serum 25(OH)D (25-hydroxyvitamin D kits, Roche Diagnostics; Roche Cobas 6000 analyzer) concentrations were determined by the electrochemical luminescence method. FBG (blood glucose kits, Beijing Leadman; BeckmanDXI800 access immunoassay system) was measured by the oxygen electrode method. HbA1c(D-10 glycosylated hemoglobin kits, Bio-Rad; Bio-Rad DiasTAT glycosylated hemoglobin analyzer) was measured by High Performance Liquid Chromatography(HPLC). Serum calcium (calcium kits, Ningbo Medical System; Siemens ADVIA 2400 automatic biochemical analyzer) and serum phosphorus (phosphorus kits, Siemens China; Siemens ADVIA 2400 automatic biochemical analyzer) were measured by the ion selective electrode method.

HOMA-IR was calculated from fasting insulin and fasting glucose. HOMA-IR had the formula of fasting glucose (mmol/L) × fasting insulin (pmol/L)/22.5 [23]. eGFR was calculated by the MDRD GFR equation[24]. As this was a retrospective study, and the data were anonymously analyzed, informed consent was unnecessary. The formula for calculating the product of calcium and phosphorus [$(mg/dL)^2$] is blood calcium (mmol/L) multiplication and blood phosphorus (mmol/L) multiplication and 12.4. The neutrophil ratio is the neutrophil count divided by the lymphocyte count.

Statistics Processing

SPSS19.0 software was used for statistical analysis. The continuous variables were expressed by mean ± standard deviation. Least-Significant Difference test (LSD-t) tests were used to compare the status of glucose metabolism and β cell function and NHL according to different concentrations of 25(OH) D. The

statistical analysis was performed using SPSS19.0 software. The continuous variables were represented by mean ± standard deviation. LSD-t tests were performed to compare 25(OH) D,NHL and islet function in GDM patients with different vitamin D concentrations. Multiple linear regression analysis was used to examine the association between serum 25(OH)D concentration and HOMA-IR, HOMA-β, respectively analyzed as the dependent variable with the other significantly associated variables [25(OH)D, eGFR, BMI, and age] as independent variables, and p value < 0.05was considered significant. This study was a retrospective study, informed consent was unnecessary.

Results

3.1 Basic characteristics of the subjects

A total of 33 subjects were enrolled, with an average age of 31.52 ± 5.26 years old. The mean 25(OH) D concentration of 33 patients with gestational diabetes was 21.40 ± 10.68 ng/mL, all blood biochemical and glucose metabolism-related indices were within the normal range (showed in Table 1). The patients with vitamin D deficiency accounted for 30.30% (10 cases), the patients with vitamin D deficiency accounted for 60.61%(20 cases), the patients with normal vitamin D status accounted for 9.1%(3 cases), the average serum PTH was 43.01 ± 17.06 ng/mL, fasting blood glucose 5.79 ± 0.78 mmol/L, HbA1c 5.95 ± 0.93%.

Table 1
Characteristics of the study population

Variables		n
age (year)[a]	31.52 ± 5.26	33
Gestational age(weeks)[b]	27.66(26.59–28.18)	33
25(OH)D (ng/mL)[a]	21.40 ± 10.68	33
Vitamin D deficiency[c]	60.61%	20
Vitamin D insufficiency[c]	30.30%	10
Normal vitamin D status[c]	9.1%	3
HbA1c (%)[a]	5.95 ± 0.93	33
FPC(μIU/mL)[a]	2.46 ± 0.94	33
FBG(mmol/L)[a]	5.79 ± 0.78	33
FINS(μU/mL)[a]	89.64 ± 33.72	33
Ne[a]	3.84 ± 1.62	33
Lym[a]	0.94 ± 0.49	33
NLR[a]	4.91 ± 2.70	33
iPTH(ng/mL)[a]	43.01 ± 17.06	33
Ca(mmol/L)[a]	2.28 ± 0.16	33
P(mmol/L)[a]	1.31 ± 0.17	33
the production of calcium and phosphorus [$(mg/L)^2$][a]	37.21 ± 5.83	33
HOMA-β[a]	58.43 ± 33.79	33
HOMA-IR[a]	58.43 ± 33.79	33
Note: a: mean ± SD, b: median [interquartile range], and c: percentage.		
HOMA-IR: homeostatic model estimates of insulin resistance, HOMA-β: homeostatic model estimatesβ,fasting blood glucose (FBG), 25-hydroxy vitamin D [25(OH) D], glycosylated hemoglobin (HbA1c), fasting insulin (FINS), fasting peptide (FPC), parathyroid hormone (iPTH), serum creatinine (SCr), blood neutrophil count, lymphocyte count, blood calcium (Ca), and blood phosphorus (P), neutrophil count (Ne), lymphocyte count (Lym), neutrophil-to-lymphocyte ratio (NLR).		

3.2 Comparison of G1, G2, and G3 of HOMA-IR and Glucose Metabolism Indices (FINS, HbA1c).

The subjects were divided into three groups according to serum 25(OH)D value: (G1) vitamin D deficiency [25(OH)D ≤ 20 ng/mL(50 nmol/L)], (G2) vitamin D insufficiency [25(OH)D value between 20 ng/mL (50 nmol/L) and 30 ng/mL (75 nmol/L)],and (G3) normal vitamin D status [25(OH)D ≥ 30 ng/mL (75 nmol/L)]. The results of the analyses comparing data for G1, G2, and G3 are given for the mean ± SD in Table 2; the mean HOMA-IR was significantly different between all the three groups, as shown in Table 2, and both HOMA-IR and FBG were lower with higher mean 25(OH)D concentrations. There was no significant difference of NLR between the three groups (P > 0.05) (showed in Table 2.).

Table 2
Comparison of parameters in G1,G2、G3 group (Table 2.)

	G1	G2	G3	P_{12}	P_{23}	P_{13}
25(OH)D(ng/mL)	12.84 ± 2.91	24.4 ± 2.08	34.35 ± 1.04	0.000	0.000	0.000
FINS(μU/mL)	86.90 ± 29.65	108.39 ± 33.13	45.43 ± 11.57	0.073	0.003	0.033
FBG(mmol/L)	6.18 ± 0.78	5.13 ± 0.70	5.37 ± 0.38	0.001	0.621	0.089
HbA1c(%)	10.08 ± 2.78	7.87 ± 2.34	5.66 ± 0.47	0.030	0.723	0.304
NLR	5.58 ± 3.00	3.45 ± 1.27	5.30 ± 2.54	0.042	0.286	0.865

3.4. Relationship between serum 25(OH)D concentration and glucose metabolism and NLR.

In the multiple linear regression analysis (shown in Table 3), 25(OH)D concentration is negatively correlated to FBG (β = -0.587, p = 0.001), adjusted to eGFR, gestational age, and age. In the multiple linear regression analysis (shown in Table 4 and Fig. 1), 25(OH)D concentration is negatively correlated to HbA1c (β = -0.408, p = 0.018), adjusted to eGFR, gestational age, and age. In the multiple linear regression analysis, vitamin D status was a predictor of HOMA-IR (β = -0.481, p = 0.007), as the dependent variable, but not eGFR, gestational age or age, as independent variables(shown in Table 5). In the 33 subjects, eGFR, gestational age and age, HOMA β is independent variable, 25(OH) D concentration as dependent variables, serum 25(OH)D concentration was objectively correlated to HOMA β (β = 0.240, p = 0.206), however, the relationship is not significant (shown in Table 6.). 25(OH)D concentration and NRL were negatively related (β = -0.389, p = 0.035), adjusted to eGFR, gestational age, and age(shown in Table 7. and Fig. 2.).

Table 3
Multiple linear regression analysis between FBG and 25(OH)D, eGFR, and age.

	FBG	
	β	*p* value
25(OH)D	-0.587	0.001
eGFR	0.274	0.080
gestational age	0.111	0.460
age	0.159	0.294

Table 4
Multiple linear regression analysis between HbA1c and 25(OH)D, eGFR, and age.

	HbA1c	
	β	*p* value
25(OH)D	-0.408	0.018
eGFR	0.345	0.733
gestational age	0.006	0.996
age	0.365	0.030

Table 5
Multiple linear regression analysis between HOMA-IR and 25(OH)D, eGFR, and age.

	25(OH)D	
	β	*p* value
HOMA-IR	-0.481	0.007
eGFR	0.055	0.738
gestational age	0.145	0.380
age	0.189	0.425

Table 6
Multiple linear regression analysis between HOMA-β and 25(OH)D, eGFR, and age.

	25(OH)D	
	β	*p* value
HOMA-β	0.240	0.206
eGFR	0.228	0.228
gestational age	-0.034	0.855
age	0.078	0.670

Table 7
Multiple linear regression analysis between NRL and 25(OH)D, eGFR, age.

	NLR	
	β	*p* value
25(OH)D	-0.389	0.035
eGFR	0.127	0.478
gestational age	-0.021	0.903
age	0.182	0.305

Discussion

Vitamin D is a fat-soluble vitamin, regulating calcium and phosphorus metabolism in *vivo*. The serum 25-hydroxyvitamin concentrations are the best index to measure the nutritional status of vitamin D in *vivo*[9]. Vitamin D, regulating bone metabolism and calcium management in bone health processes, is widely confirmed, however, its extraskeletal effects such as the immune system, regulation of cell proliferation and differentiation, and glucose metabolism got more attention either[5].

The research abroad has reported that the serum 25-hydroxyvitamin D concentration in gestational diabetes patients is lower than that of normal pregnant women, and there is an maternal elevated risk of GDM patients with vitamin D deficiency at the first trimester of pregnancy [11]. Interestingly the demand of vitamin D could be higher in pregnant women than in that without pregnant[12], which may aggravate the relative deficiency of vitamin D. In fact, 40–100% pregnant women in both the developing or developed countries, still suffer from vitamin D deficiency [11]. Some research showed vitamin D may be a potential candidate for the prevention of gestational diabetes mellitus (GDM), despite conflicting current opinions[11].

The pathogenesis of gestational diabetes,referring to the fact of vitamin D deficiency, has attracted increasingly attention. The researches show the relationship between low vitamin D status and the risk of GDM. A meta-analysis of 12 studies with 5,615 patients found a moderate correlation between 25(OH)D concentrations below 50 nmol/L of pregnant women and an increased risk of GDM (OR 1.38, 95% CI: 1.12–1.70)[13]. Moreover, Zhang found similar conclusions in a meta-analysis of 20 studies conducted in Europe, Australia, North America, and Asia with different study design including cross-sectional, case-control, nested case-control, and cohort studies including 9209 participants[1]. Vitamin D supplementation for patients with GDM seems to ameliorate different metabolic markers including blood glucose levels, insulin resistance, and inflammatory biomarkers[14] [7]. Supplementation with 50,000 International Units (IU) twice monthly has been shown to improve insulin resistance significantly, while 5000 IU daily has failed to improve blood glucose in another trial[15]. Low vitamin D status is recognized to be correlated with fasting blood glucose in prediabetes patients [16]. However, as far as we know，the information on the effect and mechanism of vitamin D on fasting glucose is limited.

In our study, 60.61% of the subjects showed vitamin D deficiency, accounting for more than half of the subjects, indicating that vitamin D deficiency was prevalent in patients with gestational diabetes. In the multiple linear regression analysis, 25(OH)D concentration is negatively correlated to FBG (β = -0.587, p = 0.001), adjusted to eGFR, gestational age, and age. In the multiple linear regression analysis, 25(OH)D concentration is negatively correlated to HbA1c (β = -0.408, p = 0.018), adjusted to eGFR, gestational age, and age. As 25(OH) D concentration decreased, fasting blood glucose and HbA1c increased, and 25(OH) D concentration was negatively correlated with fasting blood glucose and HbA1c. Vitamin D is probably a protective factor of glucose metabolism in Chinese women with gestational diabetes.

Vitamin D binds to the corresponding receptor of human islet β cells to promote insulin secretion and plays an important role in inhibiting the progression of diabetes [4]. The vitamin D status may affect blood glucose and islet function in patients with obesity [17]. Vitamin D can bind to the corresponding receptors of islet β cells or act directly on β cells to promote insulin transcription and synthesis. Consider that vitamin D deficiency may affect insulin secretion and cause insulin resistance, leading to increased fasting blood glucose and HbA1c. Vitamin D receptors (VDRs), expressed in different extra-bones peripheral tissues, were found the action on insulin receptor that promotes insulin sensitivity and insulin secretion[18] .

In the multiple linear regression analysis) of our study, vitamin D status was a predictor of HOMA-IR (β = -0.481, p = 0.007), as the dependent variable, but not eGFR, gestational age or age, as independent variable. In the 33 subjects, eGFR, gestational age, and age, HOMA β is independent variables, 25(OH) D concentration as dependent variable, serum 25(OH)D concentration was objectively correlated to HOMA β (β = 0.235, p = 0.206), however, the relationship is not significant.

There is growing evidence that inflammation plays an important role in the pathogenesis of gestational diabetes. Vitamin D may also play an important role in the inflammatory reaction, autoimmune injury, insulin secretion, and insulin resistance of islet β cells and then affect the body's glucose metabolism

[19]. Inflammation in the form of immune cells infiltrating among glandular cells can result in functional pancreatic alterations [20]. Vitamin D that can recover the physiological insulin secretion by exerting anti-inflammatory properties [21].The neutrophil to lymphocyte ratio (neutrophil-to-lymphocyte ratio, NLR) as a new inflammatory index, the value in the diagnosis and treatment of coronary heart disease, tumor, endometriosis [22]. And other diseases has attracted attention. NLR is a new inflammatory marker proposed in recent years, which is the ratio of neutrophils to lymphocytes in blood routine and can reflect the inflammatory state of the body. At present, a large number of studies have been carried out abroad on the ratio of neutrophils to lymphocytes, mainly aimed at the prognosis of tumor[23], coronary heart disease grade[22] and so on, it is found that the ratio of neutrophils to lymphocytes has a significant prognosis for the above diseases. Yilmaz, et al.[22]found a significant association with gestational diabetes. As multiple linear regression analysis can only prove the negative correlation, not further prove its causality.

Our study found that with the decrease of 25(OH) D concentrations, the NLR level increased, suggesting that with the decrease of vitamin D status may probably lead to the enhancement of inflammatory response, which may increase the fasting blood glucose level in patients with GDM. 25(OH)D concentration and NRL were negatively related (β = -0.389, p = 0.035), adjusted to eGFR, gestational age, and age. It is interesting that the negative relationship is found between 25(OH) D concentration and HOMA-IR, but not between 25(OH) D concentration and HOMA-β, which may lead to a hypothesis of vitamin D affecting glucose metabolism via insulin sensitivity, not islet function. Further research is needed to give more evidence and proof. In all, vitamin D may affect blood glucose in patients with gestational diabetes by participating in the inflammatory response.

Conclusion

The vitamin D deficiency in patients with GDM was obvious, with a decrease of 25(OH) D level, fasting blood glucose and HbA1c increased. Vitamin D may affect fasting blood glucose and HbA1c in patients with gestational diabetes by participating in the regulation of NLR inflammatory response.

Abbreviation

GDM	gestational diabetes
FBG	fasting blood glucose
25(OH) D	25-hydroxy vitamin D
FINS	fasting insulin
HbA1c	glycosylated hemoglobin
FPC	fasting peptide C
iPTH	parathyroid hormone
SCr	serum creatinine
Ca	blood calcium
P	blood phosphorus
Ne	neutrophil count
Lym	lymphocyte count
NLR	neutrophil-to-lymphocyte ratio
eGFR	glomerular filtration rate
ADA	the American Diabetes Association
BMI	bodymass index
SBP	Systolic blood pressure
DBP	diastolic blood pressure

Declarations

Availability of data and materials

All data are fully available without restriction

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

Not applicable

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Not applicable

Publication Approval

The co-authors approve this article to be published in Journal of Diabetology & Metabolic Syndrome.

Ethics approval and consent for publication

The ethics of this research was approved (ethics number 201804) and all the coauthor approve publication.

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Figures

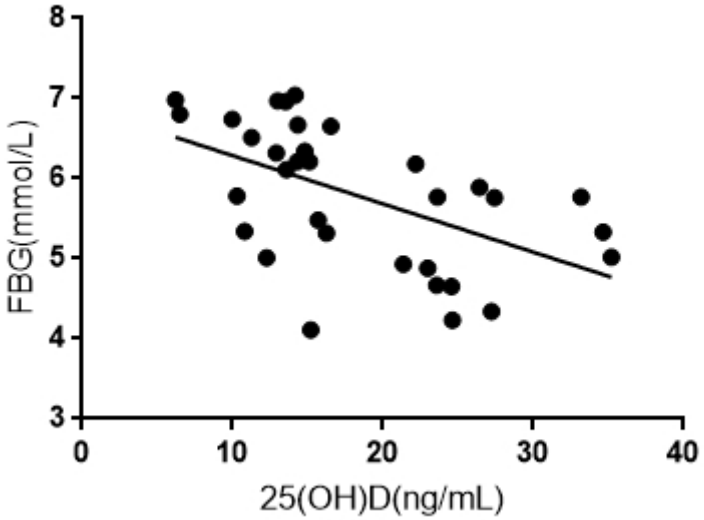

Figure 1

Inverse association between FBG and 25(OH)D (Pearson correlation = -0.539, p = 0.01)

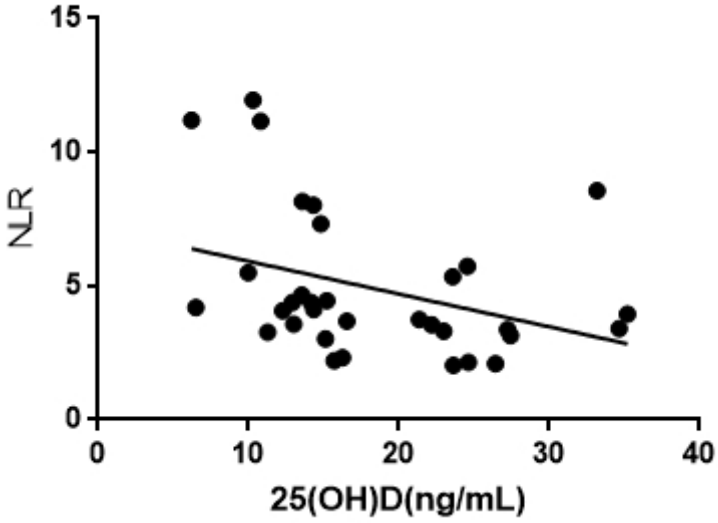

Figure 2

Inverse association between NLR and 25(OH)D (Pearson correlation = -0.353, p = 0.04)