25-Hydroxy Vitamin D Level and Its Correlation with Mean Platelet Volume in Preeclampsia

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Abstract

Background: Preeclampsia is one of the challenging complications of pregnancy, of which little is known about its etiology and pathogenesis. Many studies have shown higher mean platelet volume (MPV) in preeclamptic patients. Vitamin D deficiency is in association with larger-size platelets. Thus, we aimed to determine the correlation of vitamin D with MPV in preeclamptic patients.

Methods: This prospective case–control study was conducted in two tertiary hospitals in Tehran, Iran. Overall, 85 preeclamptic pregnant women and 85 normotensive pregnant women were entered between 2017 and 2018. Serum vitamin D concentration (ng/ml) and MPV (femtoliter) were measured for all patients.

Results: MPV was significantly higher in the cases compared to controls (10.59±1.08 vs 8.10±0.95, P=0.0001). In addition, serum vitamin D level in the preeclamptic group was significantly lower in compare to the control group (17.79±11.03 vs 30.24±12.49; P=0.0001). In multivariate logistic regression analysis, high age of mother (OR: 1.13; 95% CI: 1.01-1.27; P=0.03), low level of serum vitamin D (OR: 0.93; 95% CI: 0.87-0.99; P=0.02) and high MPV (OR: 8.83; 95% CI: 4.17-18.67; P=0.0001) were independent predictors of preeclampsia. Moreover, a correlation analysis revealed that vitamin D levels correlated negatively with MPV (r= -0.41, P<0.0001).

Conclusion: Low levels of vitamin D in preeclamptic pregnancy are associated with higher platelet activity and thrombosis. In fact, the increment of MPV level might be a potential pathway for adverse outcomes of pregnancy including preeclampsia in the context of vitamin D deficiency.

Keywords: Mean platelet volume; Vitamin D; Preeclampsia

Introduction

Preeclampsia, which is characterized by the development of new-onset hypertension and proteinuria after 20 wk of uneventful gestation, is still a leading cause of maternal and fetal morbidity and mortality (1,2). Little is known about the etiology and pathogenesis of preeclampsia; however, there have been progressions in this regard in the last decades. Inflammatory responses, endothelial dysfunction, oxidative stress and abnormal angiogenesis are some of the contributing factors in this disorder (3).

Vitamin D deficiency has been widely reported in different countries as a factor which may cause excessive inflammation and immune dysfunction, and it also may have adverse effects on hemostasis and thrombosis (4). In addition, vitamin D regulates some target genes associated with angiogenesis and fetal-placental development (5).

Recently, the vitamin D receptor has been found in platelets, playing an essential role in antithrombogenicity. Furthermore, vitamin D deficiency is in association with metabolically and enzymatically active larger-size platelets (6).

Vitamin D deficiency is a main public health problem that affects all populations of all ages in both the developed and developing world. In adults, the vitamin D deficiency plays a role in the etiology of hypertension and other adverse outcomes (4).

Low maternal vitamin D status during pregnancy has been associated with several adverse outcomes. Vitamin D deficiency is a risk factor for preeclampsia (7,8).

Preeclampsia is a serious major pregnancy-related complication. Platelets have an important role in the pathogenesis of preeclampsia (9, 10). High MPV -as a marker of more active platelets has a statistically significant relationship with preeclampsia (10, 11).

There is very little published information on the association of platelet function and vitamin D concentration. The association between high MPV and low vitamin D level was demonstrated in stable coronary artery disease patients, Korean adults, patients without chronic disease and pregnant women with gestational diabetes mellitus (6, 12-14).

We aimed to assess the relationship between serum vitamin D level and MPV value in preeclampsia.

Materials and Methods

Study design

This prospective case–control study was conducted at two tertiary hospitals affiliated to Shahid Beheshti University of Medical Sciences in Tehran, Iran between Mar 1, 2017, and May 1, 2018. The patients were selected through convenience sampling with the inclusion criteria of gestational age > 30 wk and maternal age of more than 20 years. Moreover, patients with history of valvular and coronary artery disease, diabetes mellitus, hypertension, chronic kidney and liver disease, anemia, haemoglobinopathy, heart failure with reduced ejection fraction, platelets disease, and malnutrition, active smokers, and patients consuming calcium and vitamin D supplements were excluded from the study.

Data collection

According to inclusion and exclusion criteria, 85 pregnant women with preeclampsia and 85 normotensive pregnant women admitted for termination of pregnancy in each of the study hospitals were entered into the study as case and control groups respectively. Preeclampsia was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on two occasions with a 4-hour interval and proteinuria of more than 0.3 gr in 24-hour urine sample or albumin to creatinine ratio of more than 30 mg/mmol (15, 16).

The medical records and demographic data of all the patients were reviewed and recorded for obstetrics history (i.e., age, parity, and gestational age). Maternal weight and height were measured at the sampling time, and body mass index (BMI) was calculated as kg/m2. For the assessment of vitamin D and MPV, two blood samples were taken via antecubital vein. Serum 25-OH-vitamin D (ng/ml) and MPV (femtoliter) were measured in a single laboratory. Serum 25 (OH) D3 was measured with ELISA method (EUROIMMUN®, D-23560 Lubeck, Germany). In addition, MVP assessment was conducted as a part of complete blood count (CBC). For evaluating CBC, blood samples were kept in tubes containing Ethylenediaminetetraacetic acid (EDTA) and were analyzed by automatic cell counter (Sysmex®, SE 9500; Roche Indianapolis, United States).

Statistical analysis

Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were reported as numerical (percentage) values. The normality of distributions was checked by the Kolmogorov–Smirnov test. Continuous variables were compared using an independent Student's t-test or a Mann-Whitney nonparametric test as indicated. Furthermore, Pearson's correlation coefficient was used for evaluating the relationships between MPV and vitamin D level.

For multivariable modeling, to estimate odds ratios and 95% confidence intervals for the association between each factor and preeclampsia, all variables showing a *P*-value less than 0.1 during the bi-variable correlation analysis were included into the binary logistic regression analysis model. In addition, vitamin D levels were dichotomized to normal vitamin D levels and vitamin D deficiency according to the standard cut point of 20ng/ml (18). Furthermore, to compare the rela-

tionship between variables and preeclampsia, parameters showing a *P*-value less than 0.1 during the bi-variable correlation were included into the binary logistic regression analysis model and the binary vitamin D was used instead of its continuous values (Model 2).

Ethics approval

The study protocol was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran with the registration No: IR.SBMU.RETECH.REC.1397.636.

Results

Demographics

The maternal age in preeclampsia and normotensive groups was 30.99±6.39 and 28.66±4.84, respectively. Characteristics of patients with and without preeclampsia are presented in Table 1.

Table 1: Patients characteristics in control and case groups

Variables	Control group (n=85)	Case group (n=85)	P-value
Age (yr)	28.66±4.84	30.99±6.39	0.007
Gravida	2.22±1.12	2.24±1.32	0.9
Pregnancy age(weeks)	38.31±2.21	37.44±2.31	0.01
WBC count(×10³/μL)	14.87±4.26	15.34±17.95	0.8
Neutrophil (%)	81.98±10.17	82.82±7.24	0.5
Lymphocyte (%)	14.43±6.12	16.24±10.03	0.1
Platelet (×10³/μL)	190.28±45.34	190.68±49.00	0.9
MPV (femtoliter)	8.10±0.95	10.59±1.08	0.0001
Vitamin D(ng/ml)	30.24±12.49	17.79±11.03	0.0001
BMI (kg/m²)	24.77±4.62	27.56±5.82	0.001

Data express as mean±SD

BMI: Body Mass Index; MPV: Mean Platelet volume; WBC: White blood cell

There were significant differences in characteristics including age, BMI and gestational age at delivery between two study groups; however, parity was not significantly different (*P*= 0.9).

Laboratory data

The values of laboratory variables in case and control groups are demonstrated in Table 2. According to laboratory data, regarding white blood cell (WBC), neutrophil, lymphocyte and platelet

count, there was no significant difference between case and control groups. Although, MVP was significantly higher in the cases compared to controls (*P*=0.0001). In addition, in the preeclamptic group, serum vitamin D level was significantly lower in compare to the control group. Moreover, correlation analyses revealed that 25 (OH) vitamin D levels correlated negatively with MPV (r= -0.41, *P*<0.0001).

Table 2: Multivariate analysis for the association with preeclampsia

Variable	Model 1			Model2		
	Odds ratio	CI	*P*-value	Odds ratio	CI	*P*-value
Age(yr)	1.13	1.01-1.27	0.03	1.17	1.03-1.32	0.01
Gestational Age (weeks)	0.87	0.69-1.11	0.2	0.87	0.68-1.10	0.1
MPV(femtoliter)	8.83	4.17-18.67	0.0001	8.65	4.11-17.20	0.0001
Vitamin D (ng/ml)	0.93	0.87-0.99	0.02		-	
BMI(kg/m$^{2)}$	1.12	0.97-1.30	0.1	1.12	0.97-1.29	0.1
Vitamin D deficiency		-		5.37	1.20-24.07	0.02

Data express as mean±SD, BMI: Body Mass Index; MPV: Mean Platelet volume

Logistic regression analysis

In a multivariate logistic regression analysis, high age of mother (OR: 1.13; 95% CI: 1.01-1.27; *P*=0.03), low serum vitamin D level (OR: 0.93; 95% CI: 0.87-0.99; *P*=0.02) and high MPV (OR: 8.83; 95% CI: 4.17-18.67; *P*=0.0001) were presented as independent predictors of preeclampsia (Table 2). While 53 patients (62%) with preeclampsia had vitamin D deficiency, only 9 (10.5%) normotensive patients had vitamin D level less than 20 (*P*=0.0001). In the model of multivariate analysis of the binary vitamin D level, vitamin D deficiency was associated with 5-fold increase of preeclampsia, even after adjusting for other variables (Table 2).

Discussion

In the current study, low serum vitamin D levels and high MPV were more common in patients with preeclampsia in compare to the control group. Furthermore, vitamin D deficiency was associated with 5-fold increase of preeclampsia, even after adjusting for MPV and other risk factors. There was a negative correlation between vitamin D level and MPV in pregnant women. To the best of our knowledge, this is the first study to assess this correlation in preeclampsia.

In this study, maternal age and BMI were associated with preeclampsia and the preeclamptic group had a higher BMI and age than the normotensive group. These results are consistent with previous studies (17-19).

Although the major number of women keep a normal level of platelet count throughout a normal pregnancy, the normal range of platelet counts decreases. The increase of blood volume, platelet activation, and platelet clearance all lead to a "physiologic" decrease in the platelet count. Moreover, platelet count and MPV show an inverse relationship. Therefore, the MPV increases minimally during pregnancy (20,21).

Platelet activity increases in pregnancy; in fact, in preeclamptic women, primary endothelial injury and inflammatory processes can increase platelet turnover and deteriorate the platelet characteristics (10, 11). MPV, as a parameter providing information on platelet velocity and activity, can be increased or decreased according to the severity of inflammatory process (12). Although there are controversies in the utility of MPV in predicting preeclampsia, many studies have shown higher MPV in patients with preeclampsia.

The increasing of platelet turnover caused by endothelial damage decreases the platelet count and increases the MPV in preeclamptic females (22). Severe preeclamptic pregnant women had a significantly higher MPV (23). In our study, MVP was significantly higher in the cases compared to controls.

In line with the current study, the increase of MPV value may play an important role in predicting the risk of preeclampsia (22). In addition, the MPV of the early, late preterm and term preeclamptic women was statistically higher than that of the normotensive pregnant women (10).

In another study, among the blood coagulation parameters and platelet indices the thrombin time was the best marker for early diagnosis of preeclampsia while MPV was the potential marker of its severity (24). On the other hand, some studies failed to confirm any significant correlation between MPV and preeclampsia or the severity of it (25, 26). Due to the difference in technologies used, the results of measuring the MPV give different. Therefore, conflicting results on MPV values are most possibly due to the differences between the equipment and/or methods used (25).

Vitamin D deficiency may increase oxidative stress and antiangiogenic factors in a pregnant woman and predispose her to an inflammatory response, which may lead to endothelial dysfunction, the main factor in preeclampsia. Furthermore, vitamin D regulates key target genes associated with angiogenesis and fetal-placental development, which are important factors in the pathophysiology of preeclampsia (5). This study revealed a significant independent correlation between vitamin D deficiency and preeclampsia. There are some studies in line with this study. Vitamin D level at the late pregnancy was significantly lower in preeclamptic mothers (7). While two studies demonstrated that maternal vitamin D deficiency at the early gestational age and before the third trimester were associated with an increased risk of preeclampsia (3, 8), two other studies didn't find any difference in the vitamin D level before the third trimester of pregnancy between preeclampsia and control groups (27, 28). Three studies evaluated the vitamin D level at different gestational ages, but none of them detected any predictive value for vitamin D deficiency in early stages of pregnancy; however, one study revealed lower vitamin D level at 24–26 wk of gestation was associated with a significantly increased risk of preeclampsia (3, 29, 30). Data supporting the effect of vitamin D supplements usage in pregnancy for prevention of preeclampsia also remains controversial (31,32). Consistent with this study, a recent meta-analysis of 23 studies revealed that pregnant women with vitamin D deficiency at cutoff of 20 ng/ml were at more risk of preeclampsia (33).

Studies have conflicting results on the relation between MPV values or vitamin D level and preeclampsia. Such results in the literature may be explained by a number of factors. Contradictory results on vitamin D may be explained by different study designs (case-control and other designs), vitamin D cut-off concentrations, gestational age at sampling, and vitamin D quantification methods. In previous investigations on potential adverse effects of MPV on preeclampsia, vitamin D deficiency has not been considered in the analyses. This study revealed that not only vitamin D deficiency and high MPV correlated with preeclampsia independently, but also there was a negative correlation between vitamin D level and MPV in pregnant women. The association between MPV level and nutritional or inflammatory markers has been investigated in previous studies and at least two recent studies support the association between low vitamin D level and high MPV (6, 13, 34). Although the effect of vitamin D on preeclampsia is probably multifactorial, this study provided further evidence linking vitamin D to a higher platelet reactivity and thrombosis.

This study had several limitations. First, there was a seasonal change in vitamin D concentration due to different sun exposure. We measured serum vitamin D concentration only before delivery and not during the pregnancy. However, vitamin D deficiency at the first trimester is not associated with subsequent preeclampsia risk. Second, study population was very hydrogenous. Patients with different parity and risk factors were enrolled in this study. These factors were not analyzed due to limited sample size. Further large-scale studies are needed to confirm our result and also investigate other factors.

Conclusion

Vitamin D deficiency was associated with high MPV but both of these parameters independently correlated with the risk of preeclampsia. This

should be investigated in further studies whether vitamin D supplement can decrease this risk.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conflict of Interest

The authors declare that they have no competing interest.

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