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Maternal Serum Vitamin D Level in Preeclampsia Versus Normotensive Pregnant Females: A Comparative Study

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Abstract

Background: **Preeclampsia is a severe medical disorder affecting pregnant women. Vitamin D was thought to play an essential role in its pathogenesis.** ***Aim:*** **To evaluate the association between vitamin D and preeclampsia.** ***Subjects and Methods:*** **This case-control study was conducted at the obstetrics and gynecology department at Suez Canal University Hospital. We recruited patients admitted to the ward diagnosed with mild or severe preeclampsia and another group of normotensive women as a control one. They were subjected to complete history taking, blood pressure measurement, urine analysis to detect proteinuria using a dipstick screening strip, and vitamin D analysis. Neonatal serum calcium was measured.** ***Results:*** **There was a significant difference in vitamin D levels which was significantly decreased in women with severe PE (p-value < 0.001). Women with severe preeclampsia were delivered by cesarean section than their peers (p-value 0.017). The neonatal total serum calcium level was significantly lower among preeclampsia groups (p=0.003). Also, the birth weight of the neonates was significantly lower among preeclampsia groups (p<0.001).** ***Conclusion:*** **Vitamin D deficiency affects a significant proportion of pregnant women. Vitamin D deficiency was associated with the occurrence of PE.**

Keywords: Vitamin D; Preeclampsia; Neonatal hypocalcemia

Introduction

Preeclampsia (PE) is a multiorgan disorder characterized by elevated blood pressure and proteinuria in pregnancy. It affects about 3-5% of pregnant ladies and leads to the affection of the kidneys, liver, retina, blood components, and the uteroplacental unit[(1)]. It occurs after 20 weeks of gestation and may lead to severe complications such as elevated liver enzymes, hemolysis, low platelet count (HELLP syndrome), and seizures (eclampsia). Risk factors for developing PE include young or advanced maternal age, nulliparity, obesity, family history, autoimmune diseases, and history of the underlying hypertensive disease[(2)]. PE results from the exaggerated innate

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immune reaction that leads to a severe inflammatory reaction, vasoconstriction, and placental and renal impairment[3]. Vitamin D3 calciferol deficiency has been implicated as a contributing factor in maternal and fetal adverse events. Recently, a non-traditional function for Vitamin D3 has been studied extensively. It has been reported that vitamin D3 plays a vital role in innate and adaptive immunomodulation. This raises significant concerns about the association between vitamin D3 deficiency and PE[4]. Variable results were reported about the association between vitamin D and PE[5, 6, 7]. Early detection of vitamin D deficiency, which is a modifiable risk factor, would be paramount in preventing PE[1]. This study was conducted to evaluate the association between vitamin D and PE. The primary objective was to evaluate the level of vitamin D in preeclamptic women and normotensive women. The secondary objective was fetal outcomes such as birth weight, stillbirth, neonatal complications, neonatal intensive care unit (NICU) admission, and neonatal serum calcium levels.

Subjects and Methods

This case-control study was conducted at the obstetrics and gynecology department at Suez Canal University Hospital from June 2018 to January 2021. We recruited patients admitted to the ward following inclusion and exclusion criteria. The inclusion criteria were a) age from 18- 35 years, b) the first time to have PE (defined as elevated blood pressure ≥ 140/90mmHg and proteinuria in urine +1 or more by dipstick after 20 weeks gestation (8)), c) singleton and a viable fetus, d) no history of medical disorders but for hypertension, e) no other complications during pregnancy, and f) gestational age from 34- 40 weeks. The exclusion criteria were a) women with pre-existing medical disorders, b) a history of drugs that affect vitamin D and calcium metabolism as antiepileptic drugs, theophylline, and anti-tuberculous drugs in the last six months, c) congenital fetal anomalies, and d) women with known thrombophilia. Eligible women for the study were classified into three groups: mild PE, severe PE, and normotensive women (these were recruited as women aged 18-35yrs, normotensive women, any parity, with singleton pregnancy, with no history of medical disorders, presented to the outpatient clinic or the labor and delivery ward for any complaint or in labor from 34- 40 weeks gestation). Each group included 30 patients. They were subjected to the following: Complete history taking about personal, menstrual, obstetric, and chronic illness if present. Examination with a focus on body mass index (BMI), and blood pressure measurement. Urine analysis to detect proteinuria using a dipstick screening strip. Laboratory investigations: complete blood count (CBC), liver enzymes, serum creatinine, and Proteinuria. Laboratory tests were done as routine investigations for diagnosis by automated machines; CBC by Sysmex XP-300 machine, (ALT, AST & creatinine) was done by Dimension RXL MAX machine with factory kits named Siemens and proteinuria was estimated by Mission expert (strip urine) named Acon.

Vitamin D assessment

Two blood samples were collected (3ml) from the mother. Clotting was allowed, and the sample was centrifuged for 10 minutes at

5000 rpm. The serum was stored in Eppendorf tubes at -20◦C until the samples were collected. Serum vitamin D was assayed using VD220B ELISA kits and a machine named the Infinite F50 Robotic. Vitamin D levels were classified as sufficient (≥ 30/ml), insufficient (20-30 ng/ml), and deficient (<20 ng/ml)[9].

Neonatal measures

serum calcium was measured for each newborn. A 3ml blood sample was withdrawn immediately after birth, clotting was allowed, then centrifuged for 10 minutes at 5000 rpm. The serum was examined immediately for total serum calcium levels by an automated machine; Cobas C311. Hypocalcemia was defined as a serum calcium level <8mg/dl and <7mg/dl in term and preterm neonates, respectively[10].

Sample Size

The sample size was calculated using the Stata program, setting the type-1 error (α) at 0.05 and the power (1-β) at 0.8. Previously, the mean vitamin D level among preeclamptic women was (9.7± 4.9ng/ml) while among controls was (14.8± 6.6ng/ml). Women with mild preeclampsia (9.4± 5.6ng/ml) had similar results as severe preeclampsia (P=0.811)[11]. Calculating these values produced a minimal sample size of 25 cases in each group with a 15 % drop-out rate; the total sample size was 30 cases per group.

Ethical Considerations

The study was approved by the research ethics committee of the Faculty of Medicine, Suez Canal University on 16/5/2018. (Ref no. 3485). All patients signed informed written consent before recruitment.

Statistical analysis

Collected data were processed using SPSS program 23 (statistical package for social science). Quantitative data were expressed as mean ± SD while Qualitative data as numbers and percentages. Student test was used to test the difference between quantitative variables and Chi-Square test was used to test the significance of difference for qualitative variables. A probability value (p-value) <0.05 was considered statistically significant. A cut-of-point and ROC curve were used to test the validity of maternal vitamin D levels in diagnosing mild and severe preeclampsia.

Results

There was no statistically significant difference in maternal age, GA, parity, and BMI between the study groups (p>0.05). Blood pressure measurement among the three groups was provided (Table 1). There was no statistically significant difference in the laboratory values of each group. A significant difference was noted in proteinuria levels (p-value < 0.001). Also, there was a significant difference in vitamin D levels which was significantly decreased in women with severe PE rather than those with mild PE and the control group (15.67 ± 8.25, 17.53 ± 6.93, and 23.57 ± 8.11, respectively, p-value < 0.001) (Table 2). Caesarian delivery was evident in women with more severe PE than their peers (p-value 0.017). Maternal care unit admission was significantly higher among pre-eclampsia groups (p<0.001). The neonatal total serum calcium level was significantly lower among preeclampsia groups (p=0.003). Also, the birth weight of the neonates was significantly lower among preeclampsia groups (p<0.001). Neonatal ICU admission was significantly higher among preeclampsia groups (p=0.012) (Table 3). The maternal serum level of

vitamin D of 15.0 ng/ml has a Sensitivity and specificity of 53.3 % & and 32.5 % respectively to predict the occurrence of maternal mild preeclampsia but with insignificant value, while the maternal serum level of vitamin D of 9.5 ng/ml has a Sensitivity & Specificity of 73.3% & 50.0% respectively to predict the occurrence of maternal severe preeclampsia but with also insignificant values. Maternal serum vit. D had an insignificant role in the prediction of mild or severe PE (P=0.09 and 0.32, respectively) (Table 4, Fig. 1) & (Fig. 2).

Table 1: Descriptive data of the studied population regarding each group at the time of delivery

	Group 1	Group 2	Group 3	P value
Age (years)	24.07 ± 4.98	24.63 ± 5.34	24.9 ± 4.76	0.510
Gestational age (weeks)	37.93 ± 1.28	37.47 ± 1.17	36.93 ± 1.29	0.147
BMI (kg/m2)	34.15 ± 4.12	31.68 ± 3.52	31.34 ± 2.71	0.125
Systolic BP (mmHg)	110.67 ± 8.28	146.5 ± 5.44	165.33 ± 6.81	**<0.001**
Diastolic BP (mmHg)	68.67 ± 6.81	90.33± 6.43	101.17 ± 4.86	**<0.001**

Data are expressed as Mean ± SD, BMI: body mass index

Table 2: Laboratory results of the studied population regarding each group:

	Group 1	Group 2	Group 3	P value
Serum creatinine (mg/dl)*	0.88 ± 0.11	0.92 ±0.086	0.94 ± 0.05	0.522
ALT (U/L)*	16.8 ± 8.88	15.43 ± 9.16	17.57 ± 8.13	0.321
AST(U/L)*	16.77 ±10.12	16.40 ± 9.92	15.70 ± 7.74	0.625
PLT (No× 103/mm3)*	236.40 ± 87.73	247.2 ± 82.84	220.1 ± 81.42	0.142
Proteinuria				
Nil	30 (100%)	0	0	**<0.001**
+1	0	30 100%)	0	
+2	0	0	6 (20%)	
+3	0	0	24 (80%)	
25(OH) vit D Level (ng/ml)*	23.57 ± 8.11	17.53 ± 6.93	15.67 ± 8.25	**<0.001**
Categories of vitamin D N (%)				
≥ 30 ng/ml	9 (30%)	2 (6.7%)	2 (6.7%)	**<0.001**
20 - <30 ng/ml	16 (53.3%)	19 (63.3%)	10 (33.3%)	
<20 ng/ml	5 (16.7%)	9 (30%)	18 (60%)	

**Data are expressed as Mean ± SD, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, PLT: Platelet*

Discussion

The mean vitamin D level was either insufficient or deficient in the studied population. This was reported as a common health problem affecting pregnant women[12,13]. This was rendered to different geographic distributions of the recruited patients, different ethnic groups, inadequate exposure to sunlight, and low vitamin D intake during pregnancy[14]. There was a significant difference in vitamin D levels among women with PE and normotensive ones. Vitamin D levels were

significantly lower among women with severe PE. This was emphasized by the previous results where vitamin D levels were significantly reduced among primiparous women with PE[11]. Additionally, vitamin D levels were reduced in women with PE at the termination of pregnancy[4, 5].

Table 3: Comparison of Maternal and fetal outcomes between the study groups

	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	P value
Mode of delivery *Normal vaginal* *Cesarean Section*	15 (30%) 15 (20%)	13 (26.7%) 17 (16.7%)	5 (3.3%) 25 (13.3%)	0.017
Maternal Care unit admission (> 2 days)	0 (0%)	3 (10%)	15 (50%)	**<0.001**
Neonatal Ca level (mg/dl)*	9.19 ± 1.22	9.18 ± 1.46	8.17 ± 1.11	**0.003**
Birth weight (g)*	3878.67± 351.1	3266.1 ±214.7	3113 ± 201.5	**<0.001**
NICU admission	1 (3.3%)	6 (20%)	11 (36.7%)	**0.012**

Data are expressed as Mean ± SD,* ***NICU:* *Neonatal intensive care unit*

Table 4: Validity of maternal vitamin D level in diagnosing mild and severe preeclampsia

	The area under the curve	*Std. Error*[a]	*Asymptotic Sig.*[b]	*Asymptotic 95% Confidence Interval*		*Cut off point*	*Sensitivity*	*Specificity*
				Lower Bound	Upper Bound			
Mild	0.331	0.062	0.091	0.209	0.452	15	53.3 %	32.5 %
Severe	0.436	0.061	0.325	0.316	0.556	9.50	73.3 %	50.0 %

However, contradictory results reported insignificant differences in vitamin D levels between hypertensive and normotensive participants, while another reported an increased risk of developing PE among women with low vitamin D levels[16, 17]. However, a negative association between vitamin D deficiency in early pregnancy and the development of PE was reported. This study recruited high-risk women as the control group[18]. Different study methods explained these conflicting results: heterogeneous populations recruited, different geographic distribution, different methods for measuring vitamin D, variable seasons, different dietary intake, and gestational age at recruitment[19]. Vitamin D acts as an endocrine hormone in addition to being a nutritive element. It has a vital role in immunomodulation. This is essential for modulating the immune tolerance in pregnant women to maintain pregnancy[20]. Additionally, vitamin D is essential for gene regulation incorporated in the placenta synthesis, leading to defective trophoblastic invasion and the development of PE[21]. Also, it has an essential role in regulating the renin-angiotensin system through the suppression of renin biosynthesis[22]. Women with severe PE had the lowest vitamin D levels and reported increased cesarean delivery rates, the lowest fetal birth weight, and the lowest neonatal calcium levels. This agreed with previous results where cesarean delivery increased in women with vitamin D[11]. Also, another

study reported an inverse relationship between vitamin D levels and cesarean delivery[23]. Contradicting results were reported by Savvidou et al.[24] as they evaluated serum vitamin D in the first trimester while its levels were altered significantly in the third trimester. It also used a different vitamin D measurement method (liquid chromatography). Increased cesarean delivery was rendered to poor uterine function in women with vitamin D deficiency[11].

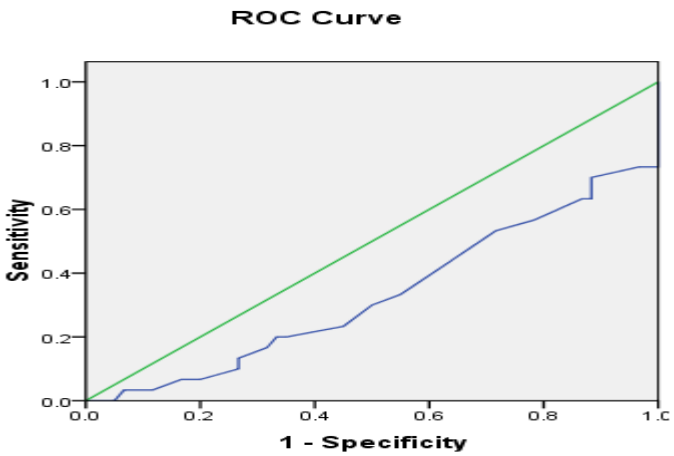

Figure 1: ROC curve of maternal vitamin D level to predict mild preeclampsia.

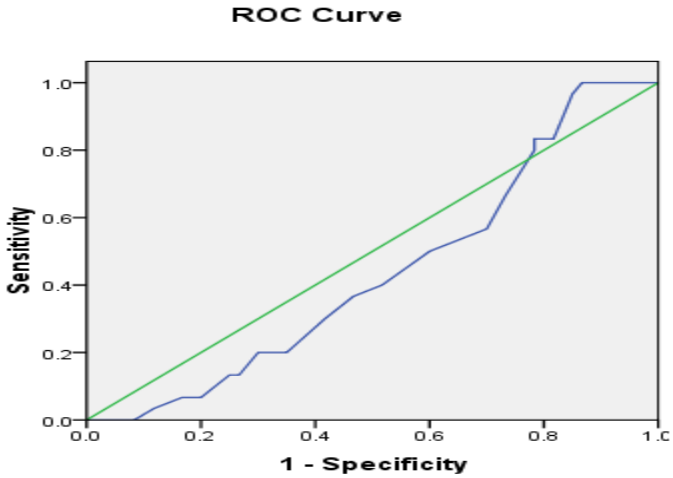

Figure 2: ROC curve of maternal vitamin D level to predict severe preeclampsia

Neonatal birth weight was significantly decreased among women with vitamin D deficiency, as reported in previous studies[11, 25]; however, contradicting results were reported[26]. A previous study reported reduced serum calcium levels in neonates whose mothers were deficient in vitamin D[27]. However, another report declared conflicting results between studies, and this was related to the different supplementation doses used in each study[28]. Strength and limitations: This study addressed a

significant issue with conflicting results in the literature. We recruited women with mild and severe PE. The small sample size is a limitation. This was a hospital-based study which limits the generalizability of the results. More extensive clinical trials are required to emphasize the role of vitamin D supplementation on maternal and fetal health.

Conclusion

Vitamin D deficiency affects a significant proportion of pregnant women. Vitamin D deficiency was associated with the occurrence of PE.

Conflict of interest: None.

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