

The relation between vitamin D level in the third trimester and preeclampsia

Original Article

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ABSTRACT

Introduction: Preeclampsia (PE) affects 2%–8% of all pregnancies and remains a leading cause of maternal and perinatal mortality and morbidity. Vitamin D was considered important for bone and calcium. There is some evidence now that low levels of Vitamin D are associated with the risk of preeclampsia but more studies are needed to prove the same.

Aim of the work: The aim of our study was to assess the relation between the vitamin D deficiency and the occurrence of preeclampsia in the third trimester of pregnancy.

Patients and Methods: This is a prospective case control study, where 90 pregnant women between 27-40 weeks of gestation were recruited from the outpatient clinic in Al zahraa university hospital, they subjected to 25(OH) vit D level estimations and transabdominal ultrasound. Follow up was done till delivery to assess occurrences of preeclampsia and assessment of fetal outcome. Cases were divided into control (n=30) and preeclampsia (n=60) groups. According to the severity, the preeclampsia cases were subdivided to mild (n=40) and severe preeclampsia(n=20)..

Results: The two groups (PE and Control) were comparable regarding maternal age, BMI and gestational age. There was significant relation between deficient 25-(OH) vitamin D and BMI. Patients with severe preeclampsia had significant lower level of 25-OH D than the mild preeclampsia and normotensive patients. There was no significant difference in 25(OH)vit D level between mild preeclampsia and control group.

Conclusion: there were significant association between vitamin D deficiency and severe preeclampsia. Vitamin D deficiency may be a risk factor for preeclampsia and may be helpful for prediction of preeclampsia and its severity.

Key Words: Deficient 25-OH vit D, 25(OH) vitamin D, preeclampsia.

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INTRODUCTION

Vitamin D is a steroid hormone that is derived primarily from the synthesis in the skin through exposure to ultraviolet B radiation. Vitamin D undergoes hydroxylation in the liver to form 25 hydroxy vitamin D [25(OH) vitamin D]^[1].

Maternal vitamin D deficiency is a common public-problem. Staggering rates of poor vitamin D status are found among pregnant mothers throughout the world^[2].

Vitamin D deficiency is defined as serum 25(OH) D of less than 25 nmol/l (10 ng/ml). Approximately one billion people worldwide are estimated health deficient with people living in Europe, the Middle East, China and Japan at particular risk^[3]. The high prevalence of vitamin D deficiency during pregnancy is increasingly recognized, especially in dark women, in northern latitudes and during winter season^[4]. The pathogenesis of

PE involves a number of biological processes, there are several hypotheses to suggest how Vitamin D levels may affect these processes. Vitamin D's role in modulating pro-inflammatory responses and decreasing oxidative stress in PE, promoting angiogenesis through vascular endothelial growth factor(VEGF) and gene modulation, and decreasing blood pressure through the renin-angiotensin system (RAS)^[5]. Vitamin D has immunosuppressive effects. Vitamin D deficiency is reported to be associated with increased secretion of proinflammatory cytokines in healthy women. In vitro studies have shown that 1, 25(OH)₂ D₃ could modulate IL-6 and TNF- α expression by suppressing NF- κ B^[6]. It is also found that vitamin D inhibits activation and proliferation of T cells and stimulates the IL-10 secretion and T- regulatory cell production, which are vital in maternal immune tolerance for normal placenta implantation^[7]. There is a growing interest in the role of maternal vitamin D status in the

pathophysiological development of preeclampsia. It has been reported that serum levels of inflammatory cytokines, such as IL-6, TNF- α and IL-10, are obviously elevated in women with preeclampsia^[8]. Maternal vitamin D deficiency is associated with a 5-fold increase in the odds of preeclampsia compared with normotensive controls^[9]. Maternal effect of hypovitaminosis D have been reported to increased preeclampsia and gestational diabetes, increased cesarean section rate and hyperemesis gravidarum^[10].

AIM OF THE STUDY

The aim of our study is to study the relation between maternal vitamin D status and the occurrence of preeclampsia in the third trimester.

PATIENTS AND METHODS

This is a prospective case –control study, that was conducted at Al zahraa University Hospital in the period between May 2018 and September 2018.

Ethical Aspects :

The nature of the study and the aim of the study were be illustrated to all participants, Informed verbal consent was obtained from each woman before participating in the study. Every recruited women had the right to withdraw from the study without being adversely affected regarding the medical service she should be receive.

Ethical approval was obtained from Ethical Committee of Obstetrics and Gyneacology department of Al Zahraa University.

Study population:

This study included 90 pregnant women of 27-40 weeks of gesataion (confirmed by early ultrasound). Cases were recruited from outpatient clinic of Al Zahraa University Hospital after fulfillment of the inclusion criteria (primigravida, singleton pregnancy). The exclusion criteria were fetal congenital anomalies, chronic illness (as: chronic hypertension, diabetes (pregestational or gestational DM), Chronic renal and liver diseases or abnormal parathyroid hormone), pregnant women taking vitamin D supplement, and pregnant women with BMI (Body mass index) >35. All cases were divided into two groups according to blood pressure and presence of proteinuria. 1st group : preeclamptic group (60 cases, they were subdivided into 40 (66.6%) mild and 20 (33.3%) severe preeclampsia), 2nd group : 30 healthy normotensive pregnant control group (average blood pressure measurement , no proteinuria) .

Methods

All women in the study was submitted to: Complete history taking , history of the current pregnancy (to assess presence of headache, epigastric pain, blurring of vision, fetal kicks, history of hyperemesis in the 1st trimester.

General examinations were done epically, measurement of weight ,height and calculation of body mass index (BMI), vital signs: pulse, Blood pressure measurement : in semi sitting comfortable position, the arms should be fully supported in flat surface at heart level using adequate sized cuff and in two occasions 4-6 hours apart. Abdominal examestation and auscultation of fetal heart sound were done. Then, routine investigation were done included liver functions tests, INR,complete blood count, kidney function test, random blood sugar, protein in 24 h urine and complete urine analysis.

Ultrasound examination for assessment of fetal wellbeing, exclusion of gross anomalies, assessment of fetal Biometry assessment of placental (site, grade) and liquor volume (The equipment: LOGIQ V5 Ultrasound in the department of obstetrics and gynecology in Al-Zahraa University Hospital).

Measurement of serum 25(OH)vit D level was measured using Enzyme immunoassay (EIA) for the quantitative measurement of serum 25-OH vit D.

All women and their fetuses were observed during pregnancy until delivery and neonatal outcome were recorded included Apgar score, fetal growth, death & weight , and admission to NICU.

In our study, we compare between cases with non deficient 25 (OH) vit D ≥ 30 ng/ml (sufficient 25 (OH) vit D) and

The deficient 25(OH) vit D cases < 30 ng/ml (include both deficient <20ng/ml and and insufficient level 20-29 ng/ml) in preeclamptic and normotensive women

Statistical analysis:

Data are collected thorough history, basic clinical examination, laboratory finding and outcome measures. Differences between parametric quantitative independent groups test by t test. *P* value was set at <0.05 for significant result and <0.001 for high significant result. Data were collected and submitted to statistical analysis.

RESULTS

Table 1 shows that both groups were matched as regard to maternal age , gestational age (GA), and BMI. There was a highly significant changes in the blood pressure between the two groups ($p < 0.001$).

There was a statistically significant relation between obesity and deficient 25(OH) vit D (Table 2).

There was significant difference in the serum level of 25(OH) vit D between mild , severe preeclampsia and control groups. Serum 25(OH) vit D level was significantly lower in cases of severe preeclampsia than mild preeclampsia and control groups (Tables 3-6).

Table 1: The demographic characteristics data of the two groups.

| Variable | | Groups | | T | T-Test | |
|-------------------------|----------|----------------|---------------|--------|---------|------|
| | | Preeclampsia | Control | | P-value | Sig |
| Age(years) | Range | 20-35 | 20-32 | 0.704 | 0.483 | (NS) |
| | Mean ±SD | 26.383±3.701 | 25.800±3.718 | | | |
| Gestational Age (Weeks) | Range | 27-38 | 28-38 | -1.661 | 0.100 | (NS) |
| | Mean ±SD | 31.533±2.914 | 32.633±3.057 | | | |
| SBP(mmHg) | Range | 140-170 | 100-130 | 16.497 | *0.001> | (HS) |
| | Mean ±SD | 151.333±10.651 | 114.000±8.944 | | | |
| DBP(mmHg) | Range | 80-120 | 60-90 | 10.222 | *0.001> | (HS) |
| | Mean ±SD | 95.500±8.911 | 76.000±7.701 | | | |
| BMI(kg/m2) | Range | 24.8-34 | 23.4-31 | 1.722 | 0.089 | (NS) |
| | Mean ±SD | 29.188±1.875 | 28.463±1.899 | | | |

DBP, diastolic blood pressure . SBP; systolic blood pressure
 BMI : Body Mass Index (weight in kilograms (kg) /height in meters squared m2).
 SD= Standard deviation.
 t.test was used for quantitative variables
 p.value : level of significance .
 -p>0.05:Non significant (NS) .
 -p<0.05:Significant(S).
 -p<0.01:Highly significant(HS)

Table 2: Relation between BMI and serum level of 25(OH) vit D in all studied women

| BMI (kg/m2) | | 25(OH) vit D | | | | | | Chi-Square | | |
|-------------------|-----------|--------------|-------|----------|-------|-------|-------|----------------|---------|-----|
| | | ≥ 30ng/ml | | <30ng/ml | | Total | | X ² | P value | Sig |
| | | N | % | N | % | N | % | | | |
| Normal weight BMI | (18.5-25) | 1 | 4.76 | 2 | 2.90 | 3 | 3.33 | 0.333 | 0.564 | NS |
| Over weight BMI | (25-29.9) | 13 | 61.90 | 43 | 62.32 | 56 | 62.22 | 16.071 | <0.001* | HS |
| Obese BMI | (30 -35) | 7 | 33.33 | 24 | 34.78 | 31 | 34.44 | 9.323 | 0.002* | S |

Chi-squared test for trend .
 Non -Deficient vit D ≥ 30ng/ml.
 -Deficient Vit D <30 ng/ml include : 1-insufficient vit D 20-29ng/m
 -2-deficient vit D <20 ng/ml
 BMI : Body Mass Index (weight in kilograms (kg) /height in meters squared(m2)).
 Normal weight :BMI is 18.5-2
 Overweight :BMI is 25-29.9
 Obese :BMI is 30 or more
 (American cancer society.,2015)

Table 3: Comparison of serum of 25(OH) vit D between normal pregnancy and the severity of preeclampsia

| 25(OH)vit D Level Ng/ml | Control | Severe | Mild | P value | Sig |
|-------------------------|-----------------|--------------|--------------|---------|-----|
| Range | 7.95-67.3 | 5.72-26.1 | 13.5-41.1 | <0.001* | HS |
| Mean± SD | 26.516 ± 11.062 | 12.892±6.117 | 27.720±5.570 | | |
| ≥30ng/ml N (%) | 7 (23.33%) | 0 | 14 (35.00%) | 0.001* | S |
| <30ng/ml N (%) | 23 (76.67%) | 20 (100.00%) | 26 (65.00%) | | |

ANOVA Test analysis of variance.

Table 4: Comparison between serum level of 25(OH) vit D in control and mild preeclampsia

| 25(OH)vit D Level Ng/ml | Mild | Control | P value | Sig |
|-------------------------|--------------|---------------|---------|-----|
| Range | 13.5-41.1 | 7.95-67.3 | 0.553 | NS |
| Mean± SD | 27.720±5.570 | 26.516±11.062 | | |
| ≥30ng/ml N (%) | 14 (35.00%) | 7 (23.33%) | 0.450 | NS |
| <30ng/ml N (%) | 26 (65.00%) | 23 (76.67%) | | |

unpaired T test.

Table 5: Comparison between serum level of 25(OH) vit D level in control and severe preeclampsia .

| 25(OH)vit D Level Ng/ml | Mild | Control | P value | Sig |
|-------------------------|--------------|---------------|---------|-----|
| Range | 5.72-26.1 | 7.95-67.3 | <0.001* | HS |
| Mean± SD | 12.892±6.117 | 26.516±11.062 | | |
| ≥30ng/ml N (%) | 0 (0) | 7 (23.33%) | <0.001* | HS |
| <30ng/ml N (%) | 20 (100.0%) | 23(76.67%) | | |

Table 6: Serum level of 25(OH) vit D in mild and severe preeclampsia.

| 25(OH)vit D Level Ng/ml | Severity of preeclampsia | | P value | Sig |
|-------------------------|--------------------------|---------------|---------|-----|
| | Mild | Severe | | |
| Range | 13.5 - 41.1 | 5.72 - 26.1 | <0.001* | HS |
| Mean ±SD | 27.720 ± 5.570 | 12.892 ±6.117 | | |
| ≥30ng/ml N (%) | 14 (35.00%) | 0 | <0.003* | S |
| <30ng/ml N (%) | 26 (65.00%) | 20 (100.00%) | | |

DISCUSSION

Preeclampsia is a multisystem disorder defined as new-onset hypertension and proteinuria. In developed countries, the perinatal mortality rate among preeclamptic pregnancies is five times as great as nonpreeclamptic pregnancies^[11].

There is a growing interest in the role of maternal vitamin D status in the development of preeclampsia. Vitamin D is a prohormone that is either made in the skin through ultraviolet B radiation exposure or ingested orally^[6].

Most research on vitamin D and preeclampsia has been conducted in predominantly white populations with small numbers of preeclampsia cases, and results have been inconsistent^[12].

Vitamin D deficiency in pregnant women is due to inadequate sunlight exposure, limited vitamin D-rich food sources, and the use of prenatal vitamins with low doses of vitamin D. Vitamin D has diverse and protean functions that may be relevant in the pathophysiology of preeclampsia, including abnormal placental implantation and angiogenesis, excessive inflammation, hypertension, and immune dysfunction^[13].

In the current study, 90 pregnant women between 27-40 weeks of gestation were recruited in the study, they were subjected to measurement of 25(OH) vit D level and ultrasound, then they divided to control and preeclampsia groups according to blood pressure estimation and the degree of proteinuria, the preeclampsia cases subdivided to mild and severe preeclampsia.

According to the level of 25(OH)vit D, the cases

were subdivided into group with non deficient 25(OH) vit D level ≥ 30 ng/ml and deficient 25(OH) vit D level < 30 ng/ml.

In the current study, the preeclamptic and normotensive women were comparable as regard to the maternal age, gestational age and BMI and there were no statistically differences between the groups. Although, the women who had preeclampsia are slightly elder than the control but that difference was statistically nonsignificant.

Our study found that there was a statistically significant relation between obesity and serum level of 25(OH) vit D. These results were consistent with Zhao *et al.*, (2017)^[14], who performed a cohort study and demonstrated that the serum 25(OH) D concentration was much lower in pregnant women with a pre-pregnancy BMI of ≥ 25 kg /m². Shantavasinkul *et al.*, (2015)^[15] reported that being overweight or obese might have an adverse effect on nutritional 25(OH) vit D status. Also, Zoya *et al.*, (2009)^[16] performed a study population to detect the dependency of 25(OH) vit D status on BMI and found that there is a significant decrease of 25(OH) vit D level with increasing BMI p value < 0.01 . Mrinal *et al.*, (2014)^[17], performed a cross sectional study to detect the dependency of 25(OH) vit D status on the anthropometric data as BMI and found that deficient 25(OH) vit D < 20 ng/ml is statistically related to BMI > 30 p value = 0.001.

Our study found that serum 25(OH) vit D level was lower in the preeclampsia group than the normotensive group but this is not statistically significant.

Our result was in agreement with Wetta *et al.*, (2014)^[18], who performed case control study and measured serum 25(OH) vit D level and found that mean serum 25(OH) vit D levels was not significantly different between women with preeclampsia and controls p .value = 0.46. Whereas Powe *et al.*, (2010)^[12] performed case control study on first trimester serum total 25(OH) vit D and found that total 25(OH) vit D levels were similar in the preeclampsia group and control group p .value = 0.435.

Disagree with Singla *et al.*, (2015)^[19], who measure serum 25(OH) vit D level in preeclampsia cases and normotensive cases and found that mean serum 25(OH) vit D was statistically significantly lower among preeclampsia cases (mean \pm SD 9.7 \pm 4.95 ng/ml) as compared to normotensive controls (mean \pm SD 14.8 \pm 6.68 ng/ml) p value = 0.0001.

Whereas, on studying the relation of 25(OH) vit D to the severity of preeclampsia we found there was significant reduction in the level of 25(OH) vit D in severe preeclampsia than mild preeclampsia and healthy normotensive women

The result agreed with Lisa *et al.*, (2014)^[20] who performed case cohort study at ≤ 26 weeks' gestation and concluded that Maternal 25(OH) vit D deficiency may be a risk factor for severe preeclampsia but not for its mild subtypes.

Arthur *et al.*, (2010)^[21] found that maternal 25(OH) vit D concentration was lower in women who subsequently developed severe preeclampsia compared with controls. Maternal 25(OH) vit D of less than 50 nmol/liter was associated with an almost 4-fold odds of severe preeclampsia (unadjusted odds ratio, 3.63; 95% confidence interval, 1.52- 8.65) compared with levels of at least 75 nmol/liter. They concluded that maternal 25(OH) vit D deficiency was associated with increased risk of severe preeclampsia. 25(OH) vit D deficiency may be a modifiable risk factor for severe preeclampsia.

Christopher *et al.*, (2010)^[22], measured total 25(OH) vit D levels in early onset severe preeclampsia (EOSPE) cases group and normotensive groups and found that statistically significant difference in the 25(OH) vit D levels between the two groups EOSP (18ng/ml) control group (32ng/ml) p . value < 0.001 .

In agreement with Zhao *et al.*, (2017)^[14] found that the serum 25(OH) vit D concentration was significantly lower in pregnant women who subsequently developed severe preeclampsia compared with those who did not. They concluded that maternal 25(OH) vit D deficiency was strongly associated with increased odds for severe preeclampsia.

Sima *et al.*, (2017)^[23] performed case control study on 75 healthy pregnant women and 74 pregnant women with preeclampsia (46 mild preeclampsia and 28 severe preeclampsia). They found that mean serum 25(OH) vit D level was 27.7 \pm 15.3, 22.9 \pm 15.9 and 27.6 \pm 16.6 in normal, mild preeclampsia, and severe preeclampsia groups $P > 0.05$, so 25(OH) vit D deficiency was not different between the groups. There was no association between 25(OH) vit D deficiency and preeclampsia severity in the study. The study of Sima *et al.*, (2017)^[23] was disagree with our study.

Mohammad *et al.*, (2015)^[24] found that comparison of 25(OH) vit D levels between normal primigravida women and severe preeclampsia women groups showed no significant differences ($P > 0.05$). Their results were also in contrary to our results.

CONCLUSION

We concluded that there were significant association between 25(OH) vit D deficiency and severity of preeclampsia. Vit D deficiency may be a risk factor for severe preeclampsia and may be helpful for prediction of severity of preeclampsia.

RECOMMENDATION

Early Measurements of 25(OH) vit D in all pregnant women may be a screening tool for prediction of preeclampsia. We also recommended 25(OH) vit D supplementation of pregnant women to protect them from severe preeclampsia.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

- Hollis BW (2005): circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implication for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 135(2):317-22.
- Halicioglu O, Aksit S, Koc F, Akman SA, Albudak E, Yaprak I, Coker I, Colak A, Ozturk C and Gulec ES (2012): Vitamin D deficiency in pregnant women and their neonates in spring time in western Turkey. *Paediatric and Perinatal Epidemiology*; 26: 53–60.
- Vieth R, John NH, Andrew S, and Robert H (2007): Risk assessment for vitamin D. *Am J Clin Nutr*;85:6.–18.
- Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK and Bhatia V (2005): High prevalence of vitamin D deficiency among pregnant women and their newborns in northern india. *AM J Clin Nutr* 81(5):1060-1064.
- Robinson CJ, Wagner CL, Hollis BW, Alanis MC and Johnson DD (2010): Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia *AM J Obstet Gynecol.* 203(4); 366.e1-6.
- Holick MF (2007): Vitamin D deficiency. *N.Engl. J.Med.*; 357: 266-281.
- Wei SQ, Audibert F, Hidiroglou N, Sarafin K, Julien P, Wu Y, Luo Z. C and Fraser W.D. (2012): Longitudinal vitamin D status in pregnancy and the risk of preeclampsia. *BJOG*, 119-832-839.
- McCracken SA, Gallery E and Morris JM (2004): Pregnancy –specific down –regulation of NF-KB expression in T cells in human is essential for the maintenance of cytokines profile required for pregnancy success. *j Immunol*, 172:4583-4591.
- Lai X, Minjae L, Arun J and James M (2014): The relation of hypovitaminosis D and il-6 in preeclampsia, *Am J Obstet Gynecol*: 1491-497.
- Mamta G, Arijit D, Sanjay J, Vandana S and Somosri R (2017): Vitamin D status in pregnancy and fetomaternal outcome and correlation with cord blood vitamin D *Indian J Med Biochem*, 21(1);42-48 .
- Roberts JM (1998); Endothelial dysfunction in preeclampsia. *Semin Reprod Endocrinol.*; 16(1);5-15.
- Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA and Thadhani R (2010). First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. *Hypertension*; 56(4);758-63.
- Adams SJ and Hewison M (2010): Update in vitamin D. *The journal of Clinical Endocrinology and Metabolism* 95(2).471-478.
- Zhao X, Fang R, Yu R, Chen D, Zhao J and Xiao J (2017): Maternal vitamin D Status in the late second trimester and the risk of severe preeclampsia in southeastern china. *Nutrient.* 14;9(2).pii.E138.
- Shantavasinkul PC, Phanachet P, Puchaiwattananon O, Chailurkit LO, Lapananon T, Chanprasertyotin S, Ongphiphadhanakul B and Warodomwicht D (2015): Vitamin D status is a determinant of skeletal muscle mass in obesity according to body fat percentage. *Nutrition* 31, 801–806.
- Zoya L, Alina CP, Fedon L, Sofie H and Johan M (2009): The Dependency of Vitamin D Status on Body Mass Index, Gender, Age and Season. *Anticancer Research* 29: 3713-3720.
- Mrinal PA, Subinay D and Anshuman D (2014): The Dependency of Vitamin D Status on Anthropometric Data *J Med Sci.* May-Jun 2014; 21(3): 54-61.
- Wetta LA, Biggo JR, Cliver S, Abramovici A, Barnes S and Tita AT (2014): Is midtrimester vitamin D status associated with preeclampsia and preterm birth. *Am J Perintol.* 31(6)541-6.
- Singla R, Padma G, Neelam A, Usha D and Rakesh K (2015): a case control study Relationship between preeclampsia and vitamin D deficiency. *Arch Gynecol Obstet* 291:1247–1251.
- Lisa M Bodnar, Simhan HN, Catov JM , Roberts JM, Platt RW, Diesel JC and Klebanoff MA(2014); Maternal vitamin D status and the risk of mild and severe preeclampsia. *Epidemiology* 2014 Mar ; 25(2);207-14.
- Arthur MB, Haeri S, Camargo CA, Espinol JA and Stuebe AM (2010): A nested case –control study of midgestational vitamin D deficiency and risk of severe preeclampsia. *J Clin Endocrinol Metab*;95(11)5105-9.
- Christophar JR, Alanis MC, Wagner CL, Hollis BW, Johnson DD (2010): Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol.*; 203(4):366, e361–366.
- Sima H, Neda E, Amir Z, Mohammad HK, Elham D, Zahra M, Fatemeh P and Shokohossadat A (2017): The Relationship of Vitamin D and Calcium level with Preeclampsia Severity: A Case- control Study. *Int J Pediatr*, 5(6): 42.
- Mohammad HD, Forouhari S, Ghaemi SZ, Khakshour A and Kiani Rad S (2015): Comparison of 25-hydroxyvitamin D and calcium levels between preeclampsia and normal pregnant women and birth outcomes. *Int J Pediatr* 3(6.1);1047-55. .