

## Original Research Article

### **Maternal Serum 25-hydroxy Vitamin D Concentrations and Calcium Levels in Preeclamptic Women, Sindh, Pakistan**

#### **ABSTRACT**

**Objectives:** To analyse the levels of serum 25-hydroxyvitamin D 25(OH)D and calcium concentrations in patients with pre-eclampsia, Hyderabad, Sindh, Pakistan.

**Methodology:** Cross sectional study was conducted at the Gynaecology and Obstetrics Department, LUHS, Hyderabad during the period of January 2020 to June 2020. Total 150 pregnant women were selected according to predesign Proforma and divided into two groups. 50 females were normal normotensive pregnant women as a control group, 100 were pre-eclamptic patients as case group. Obstetric history, clinical data was gathered and then 5ml of blood sample was collected from each patient. The blood samples were taken for the analysis of 25-hydroxyvitamin D or 25(OH)D and calcium levels. 25(OH)D were performed on chemiluminescence, While calcium concentration of Microlab 300. Data was analysed by using SPSS version 23. Student t-test was used for analysed of continuous variables.

**Results:** The maternal serum vitamin D and calcium levels were significantly decreased as compared to patient with preeclampsia and normotensive healthy pregnant ladies. The blood pressure also declined in case group with  $P < 0.001$ . Multinomial logistic regression showed significant odd ratio of Vitamin-D and Calcium deficiency in preeclampsia.

**Conclusion:** The present study reveals that 25-hydroxyvitamin D, and Calcium levels declined significantly in preeclamptic women, and might be biomarker of this life threatening disease.

**Key words:** Blood pressure, Calcium, Preeclampsia, Vitamin-D

#### **INTRODUCTION**

Pre-eclampsia (PE) is one of the common complication of pregnancy. PE is characterized by development of hypertension, proteinuria after 20th week of pregnancy with previously normal pregnancy.<sup>1,2</sup> The metabolic demand is increases during the progressive period of

pregnancy which is helpful for the better growth of foetus.<sup>3</sup> Proper nutrition throughout pregnancy is very important for mother and healthy foetus.<sup>4</sup> Vitamin D is a lipophilic vitamin. It is existing in two forms, Vitamin D<sub>3</sub> and Vitamin D<sub>2</sub>, with similar structures. Vitamin D<sub>3</sub> also known as cholecalciferol contributes to 95% of the Vitamin-D levels in the human circulation.<sup>5</sup> Vitamin-D deficiency affects all age groups. Its classical function is on bone and calcium homeostasis but it is also a pluripotent regulator of many biological functions.<sup>4</sup> It is a vitamin that affects billions of world population and the prevalence of Vitamin-D in Pakistan is higher approximately 53.3%.<sup>6</sup> Vitamin-D plays a vital role during pregnancy. Its deficiency not only affects on mother health but also impact on infant health. During pregnancy Vitamin-D deficiency results in complications like pre-eclampsia, gestational diabetes, intrauterine growth restriction and pre term birth is globally appreciated.<sup>7</sup> Vitamin D receptor (VDR) present in every tissue controlling by many genes. The placental components, maternal decidua and foetal trophoblast including syncytiotrophoblast express the CYP27B1.<sup>8</sup> The CYP27B1 is responsible to produce 25(OH)2D and then converted to 1,25(OH)2D. The concentration of 25(OH)2D is increased in placenta during the pregnancy.<sup>9</sup> Vitamin-D and its other related compound localized in these tissues the placenta is one of the prominent tissues among these barrier sites. During first trimester of pregnancy initially maternal serum 25(OH)2D levels increased significantly however, some studies on animal and human models indicated that this is due to presence of VDR in the placenta and exerts its functions on specific tissue at the maternal-fetal interface.<sup>10</sup> These receptors act as autocrine. Studies suggest that vitamin D is also responsible for the placental transport of calcium.<sup>11</sup> Serum calcium plays a very important role in regulation of blood pressure by stimulating the parathyroid hormone (PTH) and release of renin results in inducing vasoconstriction. Calcium might also have an indirect effect on smooth muscle.<sup>12</sup> The aim of this study was to analyse and compare the maternal serum 25(OH)2D and calcium levels in pre-eclamptic and normotensive females.

The objective of this study is to estimate the levels of serum 25-hydroxyvitamin D and calcium concentrations during preeclamptic Hyderabad women, Sindh

## **METHODOLOGY**

### **Setting:**

The study was conducted in Gynaecology and Obstetrics Department, Liaquat University of Medical & Health Sciences, Jamshoro with collaboration of Pathology Department LUMHS Jamshoro / Hyderabad during the period of January 2020 to June 2020.

### **Sample Size:**

The sample size was calculated according to Biostatistics Rao software. Pre-eclampsia was 5% by using the proportion of 95% confidence interval with 5% margin of error, the sample size was stand to be n=70. Total 150 subjects were recruited and divided into two groups. Group A: n = 50 Healthy pregnant women with normal B.P after 20<sup>th</sup> week of gestation and Group B: n = 100 patient with Pre-eclampsia after 20<sup>th</sup> week of gestation. after taking history, anthropometric parameters, collecting the blood sample from all participants of the study and also taking the consent on pre-designed Proforma.

### **Inclusion Criteria:**

Healthy pregnant women after 20<sup>th</sup> week of gestation within 20-35 years of age and pregnant women with pre-eclampsia after 20<sup>th</sup> week of gestation within 20-35 years of age.

### **Exclusion Criteria:**

Pregnant women below 20 or above 35 years, multiple Pregnancy, known hypertensive, diabetic, cardiac and renal, liver disease, smokers and any use of drug.

### **Blood Parameters:**

3 ml of Blood was collected from each participant by venopuncture into EDTA tubes under aseptic measures. The blood was centrifuged at 3500 rpm for 10 min, fractionated and transferred to eppendorf cups then stored at -20°C till required for analysis. Before the analysis, sample was first allowed to attain room temperature. The blood samples were taken for the analysis of 25(OH)D and calcium levels. 25(OH)D were performed on chemiluminescence, While calcium concentration of Microlab 300.

### **Ethical Consideration:**

The study was conducted strictly under the ethical rules after the approval from Ethical Review Committee of LUMHS Jamshoro.

### **Statistical Analysis Procedure:**

The data was entered in Microsoft Excel and analyzed on SPSS (Statistical package for Social Sciences) Version 23. Independent unpaired student t-test for comparison between cases and control was used for continuous variable. Results were presented as mean and standard deviation.

## **RESULTS**

The present study included 150 pregnant females, 33.4% were normal healthy pregnant whereas 66.6% were patients with preeclampsia. The Mean  $\pm$  SD of maternal age of control and cases was  $26.7 \pm 4.34$  year versus  $27.43 \pm 3.86$  year. The p-value of gestational age, parity and gravida of control and cases were insignificant (Table 1). The systolic, diastolic blood pressure was increased in cases group as compared to control. 25-hydroxyvitamin D and serum calcium was decreased in preeclamptic patients as compared to control with highly significant difference of ( $p < 0.001$ ), shown in Table 1.

The predictor of severe and mild preeclampsia observed by Multinomial logistic regression. The odd ratio of maternal serum 25(OH)2D deficiency and hypocalcaemia in severe and mild patients of preeclampsia shown significant difference (Table 2).

**Table 1.** The Comparison of Maternal age, Gestational age, Parity and Gravida, Blood pressure, Calcium and Serum 25-hydroxyvitamin D concentration between normal pregnant and patient with preeclampsia.

Variables	Group-A Control (n=50)	Group-B Cases (n=150)	P-value
Maternal age (Years)	$26.7 \pm 4.34$	$27.43 \pm 3.86$	NS= 0.059
Gestational Age (Weeks)	$27.5 \pm 3.96$	$26.4 \pm 3.43$	NS=0.061
Parity	$3.43 \pm 1.37$	$2.51 \pm 1.90$	NS= 0.059
gravida	$3.62 \pm 1.42$	$3.41 \pm 1.62$	NS=0.055
Systolic Blood Pressure (mmHg)	$110.2 \pm 74.4$	$189.3 \pm 68.9$	<0.001
Diastolic Blood Pressure (mmHg)	$74.2 \pm 5.74$	$96.5 \pm 25.9$	<0.001
Albumin (gm)	$0.4 \pm 4.1$	$0.1 \pm 3.1$	<0.05
Serum Calcium (mg/dl)	$8.57 \pm 1.94$	$6.51 \pm 3.64$	<0.05
25-hydroxyvitamin D (mg/dl)	$30.5 \pm 8.75$	$19.5 \pm 11.6$	<0.001

**Table 2.** Multinomial logistic regression of preeclampsia predictor, 25(OH)2D deficiency and hypocalcaemia

Variables	Odds Ratio	95% CI	p-value
<b>Mild preeclampsia</b>			
25(OH)2D	1.97	0.42-6.87	0.412
Hypo-calcaemia	1.72	0.34-7.9	0.54
<b>Severe preeclampsia</b>			
25(OH)2D	1.78	0.39-6.98	0.413
Hypo-calcaemia	6.57	1.54-28.5	0.015

## DISCUSSION

This is immense health related issue in pregnancy worldwide. Maternal serum 25(OH)2D deficiency during pregnancy is associated with adverse outcomes.<sup>13</sup> Lamminpaa et al.,<sup>14</sup> revealed that advance maternal age exhibited pre-eclampsia. one more study of Ghana found that maternal age is also a major risk factors of pre-eclampsia.<sup>15</sup> Fondjo LA et al.,<sup>16</sup> found that pre-eclampsia is commonly occurred in low gestational age group as compared to normotensive group with higher gestational age with (p <0.001). We found similar results in present study. Preeclampsia is more common in developing country<sup>1</sup>. Nutritional deficiency is one of the most important cause and exerted adverse effects on health of mother as well as fetus.<sup>17</sup> The Achkar M et al.,<sup>13</sup> reported that low Vitamin-D concentration is linked with high risks of preeclampsia, infertility and gestational diabetes mellitus. 25(OH)2D deficiency also associated with primary onset of preeclampsia with foetal growth retardation. Many researchers reported in their observational studies that significant association has been found between declined levels of maternal serum 25(OH)D results in enhance risk of preeclampsia<sup>18</sup>. We found similar results in present as mention in above studies. The odd ratio of mild and severe preeclampsia is approximately (1.97 and 1.78). In contrast to other case control studies reported that who developed preeclampsia having low levels of maternal serum 25(OH)D concentration as compare to normal pregnant ladies<sup>19</sup>. The results were constituent with present study.

Calcium is regulator of PTH, and it is associated with 25(OH)2D. Many studies have focused on correlation among calcium, 25(OH)2D and PTH with Preeclampsia.<sup>20</sup> In our study the calcium concentration is significantly declined. Hypocalcaemia can be mightly be a risk factor

of preeclampsia as a life-threatening disease. Gabbay A et al.,<sup>21</sup> revealed that complications of low level of calcium such as secondary hyperparathyroidism may play a vital role in the aetiology of preeclampsia.

Udenze IC and co-workers<sup>22</sup> reported that calcium concentration was low in preeclamptic women as compared to normal subjects. On another case control studies Ghana<sup>23</sup> revealed association of hypocalcaemia and preeclampsia. In present study we found similar results.

## CONCLUSION

The 25-hydroxyvitamin D and calcium play a vital role in pregnancy process of conception, implantation and development of placenta. The 25-hydroxyvitamin D and calcium deficiency are major causative factors of Preeclampsia.

## Recommendation

The vitamin D receptors and its histopathology should be in consideration for future studies. calcium is also very important element further studies regarding Parathyroid hormone should be done.

## References

1. Shaikh F, Shah T, Ansari S, Dahri S. To determine the role of Co-Enzyme Q10 and trace elements in patient with pre-eclampsia - a cross sectional study in Hyderabad. J Liaquat Univ Med Heal Sci. 2017;16(2).
2. Jamal B, Shaikh F, Memon MY. To determine the effects of copper, zinc and magnesium in patients with pre-eclampsia. J Liaquat Univ Med Heal Sci. 2017;16(1).
3. Shaikh F, Shah T, Memon A, Yousuf Memon M, Chanihoon G. Role of coenzyme q10 in pre-eclamptic patients. Rawal Med J. 2018;43(4).
4. Shaikh F, Lakhani H, Memon KA, Sarah B, Ansari S, Shah T, Ahmer A. Coenzyme Q10 Supplementation Reduces Oxidative Stress in Patients with Pre-Eclampsia. J Pharm Res Int. 2021 Sep 9;33(43B):146–51.
5. Shahid S, Ladak A, Fatima SS, Zaidi FA, Farhat S. Association of vitamin D levels with preeclampsia. J Pak Med Assoc. 2020 Dec 1;70(12(B)):2390–3.
6. Mustafa G, Asadi MA, Iqbal I, Bashir N. Low vitamin D status in nursing Pakistani mothers in an environment of ample sunshine: A cross-sectional study. BMC Pregnancy Childbirth. 2018 Oct 29;18(1):1–7.

7. Zeng S, Cheng X, Chen R, Wu J, Zhou J. Low level of Vitamin D is a risk factor for the occurrence of early and late onset pre-eclampsia in pregnant women. *Clin Lab*. 2020;66(6):1059–66.
8. Farajian-Mashhadi F, Eskandari F, Rezaei M, Eskandari F, Najafi D, Teimoori B, Moradi-Sharbabak M, Salimi S. The possible role of maternal and placental vitamin D receptor polymorphisms and haplotypes in pathogenesis of preeclampsia. *Clin Exp Hypertens*. 2020 Feb 17;42(2):171–6.
9. Coşkun S, Şimşek Ş, Camkurt MA, Çim A, Çelik SB. Association of polymorphisms in the vitamin D receptor gene and serum 25-hydroxyvitamin D levels in children with autism spectrum disorder. *Gene*. 2016 Aug 22;588(2):109–14.
10. Rezavand N, Tabarok S, Rahimi Z, Vaisi-Raygani A, Mohammadi E, Rahimi Z. The effect of VDR gene polymorphisms and vitamin D level on blood pressure, risk of preeclampsia, gestational age, and body mass index. *J Cell Biochem*. 2019 Apr 1;120(4):6441–8.
11. Khaing W, Vallibhakara SAO, Tantrakul V, Vallibhakara O, Rattanasiri S, McEvoy M, Attia J, Thakkinstian A. Calcium and Vitamin D Supplementation for Prevention of Preeclampsia: A Systematic Review and Network Meta-Analysis. *Nutrients*. 2017 Oct 18;9(10).
12. Santorelli G, Whitelaw D, Farrar D, West J, Lawlor DA. Associations of maternal vitamin D, PTH and calcium with hypertensive disorders of pregnancy and associated adverse perinatal outcomes: Findings from the Born in Bradford cohort study. *Sci Reports* 2019 91. 2019 Feb 4;9(1):1–9
13. Achkar M, Dodds L, Giguère Y, Forest JC, Armson BA, Woolcott C, *et al*. Vitamin D status in early pregnancy and risk of 5209 *Int J Pediatr*, Vol.5, N.6, Serial No.42, Jun. 2017.
14. Lamminpaa R, Vehvilainen JK, Gissler M, Heinonen S. Pre-eclampsia complicated by advanced maternal age: a registry-based study on primiparous women in Finland 1997-2008. *BMC Pregnancy Childbirth*. 2012;11(12):47.
15. Owiredu WKBA, Ahenkorah L, Turpin CA, Amidu N, Laing EF. Putative risk factors of pregnancy-induced hypertension among Ghanaian pregnant women. *JMBS*. 2012;1(3):62–76.

16. Fondjo LA, Tashie W, Owiredu WKBA, Adu-Gyamfi EA, Seidu L. High prevalence of vitamin D deficiency among normotensive and hypertensive pregnant women in Ghana. *BMC Pregnancy Childbirth*. 2021 Dec 1;21(1).
17. Chaurasia PP, Jadav PA, Jasani JH. Changed in Serum Calcium and Serum Magnesium Level in Pre-eclamptic VS Normal Pregnancy. *IJBAR*. 2012;3(6):511–3.
18. Ephraim RK, Osakunor DN, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy induced hypertension: a case-control study in the Cape Coast metropolis, Ghana. *BMC Pregnancy Childbirth* 2014; 14: 390. doi: 10.1186/s12884-014-0390-2.
19. Behjat Sasan S, Zandvakili F, Soufizadeh N, Baybordi E. The Effects of Vitamin D Supplement on Prevention of Recurrence of Preeclampsia in Pregnant Women with a History of Preeclampsia. *Obstet Gynecol Int*. 2017;2017
20. Gabbay A, Tzur T, Weintraub AY, Shoham-Vardi I, Sergienko R, Sheiner E. Calcium level during the first trimester of pregnancy as a predictor of preeclampsia. *Hypertens Pregnancy* 2014; 33(3): 311-21. doi: 10.3109/10641955.2013.877925.
21. Udenze IC, Arikawe AP, Azinge EC, Okusanya BO, Ebuehi OA. Calcium and Magnesium Metabolism in Pre-Eclampsia. *West Afr J Med* 2014; 33(3): 178-82.
22. Seely EW, Wood RJ, Brown EM, Graves SW. Lower serum ionized calcium and abnormal calciotropic hormone levels in preeclampsia. *J Clin Endocrinol Metab* 1992; 74(6): 1436-40.