ORIGINAL ARTICLE

Serum 25-Hydroxy Vitamin D Level in Preeclamptic and Normotensive Pregnancies

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ABSTRACT

Objective: To compare serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Physiology, Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore, in collaboration with Sir Ganga Ram Hospital and Lady Willingdon Hospital, Lahore, from March 2012 to April 2012.

Methodology: Thirty registered preeclamptic patients with systolic and diastolic blood pressure > 140/90 mm Hg on more than two occasions, 6 hours apart, and proteinuria at least 300 mg in 24-hour urine collection; and 30 normotensive uncomplicated pregnant women matched for age, gestational age, parity and BMI were included by convenient sampling technique. Vitamin D levels of less than 50 n mol/l (< 20 ng/ml) was the cutoff point. Spearman's rank correlation of vitamin D with systolic blood pressure and arterial pressure in both preeclamptic and normotensive pregnant women was presented in a tabulated form.

Results: Vitamin D deficiency was found in 95% of preeclamptic and normotensive pregnant women. The difference of vitamin D level between the two groups was not found significant. Although there was an inverse correlation between serum vitamin D and systolic blood pressure and arterial pressure in preeclamptic group, but this was not statistically significant.

Conclusion: Vitamin D deficiency does not seem to be affected by the state of preeclamptic and normotensive pregnancy. The correlation of systolic blood pressure and arterial pressure and vitamin D needs to be explored further by increasing the sample size.

Key Words: *Vitamin D deficiency. Preeclampsia. Normotensive pregnant women.*

INTRODUCTION

Since the early 1990s, interest has developed in the role of maternal vitamin D in preeclampsia.[1] Preeclampsia is a pregnancy-specific syndrome, developing after 20 weeks of gestation and presents as gestational hypertension and proteinuria.[2] Successful maintenance of pregnancy is supposed to due to immunomodulatory action of vitamin D which favours Th2 domination by causing a shift in the balance between Th1 and Th2 cytokines. In pregnancy specific tissues, such as placenta and decidua, vitamin D receptors (VDRs) and enzyme 1α hydroxylase for conversion of 25 (OH)D to 1,25 (OH)2 D are responsible for local, intracrine, paracrine and autocrine actions of vitamin D.[3] Pregnancy complications have been shown to occur by decreased activity of calcitriol because of less placental synthesis of vitamin D, which acts as a potent genetic regulator and immunosuppressant.[4]

In Pakistan, preeclampsia and eclampsia are one of the major maternal causes, responsible for death of one in 89 women.[5] Worldwide about 2% to 8% of pregnant women suffer from preeclampsia.[6] Almost 90% of the women in Pakistan are deficient in vitamin D.[7] In pregnancy, vitamin D deficiency ranges from 5% to 84% globally.[8]

Primary source (90%) of vitamin D, a secosteroid hormone, which after exposure to ultraviolet rays of sunlight, leads to endogenous synthesis in skin. Diet or supplements are secondary sources of vitamin D.[9]

Low vascular endothelial growth factor (VEGF) and increased pro-inflammatory cytokines in preeclampsia are because of their expression under the influence of vitamin D.[10] Critical role of vitamin D in the regulation of renin-angiotensin system has also been shown. Inhibition of renin-angiotensin system by vitamin D is through reduction of renin gene expression.[11] Thus vitamin D has an influence on the regulation of blood pressure.

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In normal cultured trophoblasts, vitamin D significantly inhibits expression of TNFα, so low level of calcitriol in preeclampsia may cause increased level of TNFα.[12]

In preeclamptic women, elevated TNFα has been implicated in the disease process. TNFα can enhance endothelin-1 production by regulating transcription of endothelin-1 gene. Significant long-term effects on systemic hemodynamics and arterial pressure regulation are at two- or three-fold above normal level of endothelin-1.[13]

In preeclamptic patients, vitamin D deficiency has not been well demonstrated and results of literature review are controversial. Thus apparently increase of vitamin D deficiency in Pakistan and perhaps its relation with pregnancy related outcomes and complications encouraged the researchers to undertake study to clarify the link between vitamin D levels and preeclampsia.

The objective of this study was to measure serum 25 (OH)D level in preeclamptic and normotensive pregnancies and defining any association between these.

METHODOLOGY

This cross-sectional analytical study was carried out in the Department of Physiology, Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore, in collaboration with Sir Ganga Ram Hospital and Lady Willingdon Hospital, Lahore, from March 2012 to April 2012, after taking approval from respective Ethical Review Boards of those institutions. All participants gave written informed consent before joining the study.

Thirty registered preeclamptic patients were included as cases with systolic and diastolic blood pressure > 140/90 mmHg on more than two occasions, 6 hours apart, and proteinuria of at least 300 mg in 24-hour urine collection. Thirty normotensive uncomplicated pregnant women; matched for age, gestational age, and body mass index (BMI) were included as controls by convenient sampling technique. Subjects taking vitamin D supplements and calcium or suffering from any of the chronic diseases like diabetes mellitus, pregestational hypertension, renal disease, multi-fetal pregnancy, liver disease, history of smoking, previous history of preeclampsia were excluded. Analysis of serum vitamin D was performed by Enzyme Linked Immunosorbent Assay (ELISA) at National Health Research Complex, Shaikh Zayed Hospital, Lahore. Approximately 5 milliliters (ml) of venous blood was collected from each subject under full aseptic conditions. Within 2 hours of collection when coagulation was complete, blood sample was centrifuged for at least 10 minutes to separate serum. Samples were stored at -20°C at the National Health Research Complex (FPGMI), Shaikh Zayed Hospital, Lahore, till completion of analysis of serum vitamin D.

Table I: Systolic and diastolic blood pressure and arterial pressure for preeclamptic and normotensive pregnant women (n=30).

Blood Pressure	Preeclamptic Median(IQR)	Normotensive Median(IQR)	p-value
Systolic	150 (12.5)	110 (10.0)	<0.001
Diastolic	100 (10.0)	70 (0.0)	<0.001
Arterial pressure	116 (10.8)	83 (3.3)	<0.001

Table II: Vitamin D levels for preeclamptic and normotensive pregnant women (ng/ml).

Groups	Median	IQR	p-value
Preeclamptic	14.04	10.64	0.21
Normotensive	13.8	6.92	

Table III: Correlation of vitamin D with systolic blood pressure and arterial pressure in preeclamptic and normotensive pregnant women.

Group			SBP	AP
Preeclamptic	Vit. D	Spearman's rho	-.10	-.12
		p-value	.60	.51
Normotensive	Vit. D	Spearman's rho	-.01	-.02
		p-value	.95	.90

Data were entered and analyzed using SPSS 15.0. Systolic blood pressure, diastolic blood pressure, arterial pressure and vitamin D levels of both preeclamptic and normotensive pregnant women were presented as median (inter-quartile range). Mann-Whitney U test was applied for non-parametric data. Spearman's rho (r) was calculated to determine correlation between vitamin D and systolic blood pressure and arterial pressure in both preeclamptic and normotensive pregnant women. A p-value ≤ 0.05 was considered significant.

RESULTS

These two groups were matched for age, gestational age, BMI and parity. Gestational age in both groups was between 21 - 37 weeks. Average age of both groups was 25 years. Average BMI was 22 kg/m^2. Parity and gravid status of both groups were also matched. All women were housewives with occasional exposure to sun, and of low socioeconomic status.

Preeclamptic group showed higher systolic and diastolic blood pressure and arterial pressure. Normotensive group showed significantly lower systolic and diastolic blood pressure and arterial pressure. So the difference between two groups was obviously significant ($p < 0.001$, Table I).

Vitamin D levels were lower in both groups and did not differ significantly ($p = 0.21$, Table II).

In the preeclamptic group, an inverse correlation was found between serum vitamin D and blood pressure (systolic BP $r_s = -0.10$, $p=0.60$; AP $r_s = -0.12$, $p = 0.51$) (Table III).

DISCUSSION

Acknowledging the role of vitamin D in preeclampsia, current study was done to find out and compare serum 25 (OH)D level in relevantly matched preeclamptic and normotensive pregmnant women. Comparison of vitamin D levels was done between two groups and it was observed that vitamin D levels were quite low in both groups with a median vitamin D level of 14.04 (IQR 10.64 ng/ml) in preeclamptic women; and in normotensive pregnant women median vitamin D level was found to be 13.8 (IQR 6.92 ng/ml). So, on comparison of vitamin D levels between two groups, it was observed that the difference between groups was insignificant with p-value of 0.21.

Majority, i.e. 95% of study population, had serum 25(OH)D concentrations indicative of vitamin D deficiency. This finding is consistent with a recent study of a small group of pregnant women studied in Karachi, Pakistan by Hossain *et al*.,[7] who found that almost 90% of pregnant women were vitamin D deficient. This high prevalence of vitamin D deficiency may be because we included pregnant women in this study who were not taking vitamin D supplements and calcium, with low socioeconomic status and poor diet, not fortified with calcium and vitamin D. In addition, they were housewives who spent maximum time indoor and therefore, having less sun exposure. In addition, all blood samples were collected in March and April, i.e. approximately the end of winter. So the season of sampling might have affected vitamin D levels. Higher incidence of preeclampsia in winter and a lower incidence in summer also suggests a possible role of vitamin D and sunlight.[14]

This study finding is in contrast to two other studies. Nested case control study by Bodnar *et al*.,[15] found that 55 nulliparous women (4.9% of the population cohort), who ultimately developed preeclampsia, had 2.5 times more chances of vitamin D deficiency in early pregnancy after adjusting for potential confounders including BMI. In a cohort study of over 23,000 nulliparous women (of which 5.2% suffered from preeclampsia) by Haugen *et al*.[16], women having higher vitamin D intake of 15 - 20 µg/day as compared to < 5 µg/day had a low rate of preeclampsia (odds ratio [OR] 0.76, 95% CI 0.6 - 0.95) after adjustment of potential confounders.

In 2010, Nested case control study by Baker *et al*. found that women who had < 50 nmol/l (20 ng/ml) of 25 (OH) D3 had 4-fold increase in severe preeclampsia.[17] In 2010, Shin *et al*. concluded that for optimal maternal and child health, adequate vitamin D intake during pregnancy is required. It has been shown by research that vitamin D is produced by placenta and it acts on placenta to control implantation, cytokine production, and immune response to infection.[18]

In 2011, Dror suggested that vitamin D has a role in preeclampsia because molecular pathways involved in pathogenesis of preeclampsia are under influence of vitamin D.[4] In 2011, Ringrose in a case control study of 78 women near term with two readings of blood pressure above 140/90 mm Hg and 109 controls found women more likely to have hypertension with low circulating 25 (OH)D3 concentrations.[19]

As compared to normotensive pregnant women, Robinson *et al*.[20] found low circulating 25 (OH)3 levels in preeclamptic women.

This discrepancy can be due to several reasons. In this study, serum 25 (OH)D concentrations were measured at a mean of 30 weeks of gestation compared with a mean of 10 weeks of gestation in the study of Bodnar *et al*.[15] Haugen *et al*. found more reduction in preeclampsia (OR 0.81,95% CI 0.68 - 0.97) in women who had taken vitamin D supplements for three-time points (pre-pregnancy, in early pregnancy and in late pregnancy) as compared to women who had taken supplements only in early or either late pregnancy (OR 0.87, 95% CI 0.75 - 1.02) and women who had not taken vitamin D supplements at all.[16]

It is possible that third trimester vitamin D concentration may have a less influence on placentation and preeclampsia than vitamin D concentration in the first trimester. During late pregnancy along with vitamin D insufficiency perhaps other factors, both nutritional and non-nutritional, may ultimately determine who develops preeclampsia. These may include exercise, dietary intake of calcium or long chain n-3 fatty acids.

These study findings are consistent with a few studies in which positive relationship between vitamin D deficiency and hypertension in pregnancy was not found.

Shand *et al*. in his study in British Columbia, Canada of a group of 221 pregnant women who were largely vitamin D deficient, found that rates of preeclampsia, gestational hypertension or adverse pregnancy outcome in this group was not different.[21] Powe *et al*. in nested case control study in U.S.A., when made comparison of first trimester total and free 25 (OH)D levels of women who ultimately developed preeclampsia versus normotensive pregnancies, found that levels were similar.[22] With 25 (OH)D levels < 15 ng/ml, there was a tendency towards greater risk of preeclampsia but the association was non-significant. (adjusted OR=1.35; 95% CI :0.40 - 4.50).

In this study, it was found that vitamin D levels were negatively correlated with systolic blood pressure and arterial pressure which may mean that vitamin D deficiency may lead to higher blood pressure in preeclamptic females although the results were not statistically significant. This finding is consistent with another study by Hossain *et al*. in 2011 in Pakistani mothers in Karachi.[7]

In this study, only single measurement of 25(OH)D was done in late pregnancy. Thus the evaluation of the potential role of vitamin D status in the development and progression of preeclampsia during early pregnancy was limited. Measurement of 25(OH)D in this study was done after preeclampsia had developed, so reverse causality could not be ruled out. Genetic differences in vitamin D metabolism could be the possible explanation for these inconsistent findings. Heterogeneity in effects by race and/or vitamin D receptor genotype could have been explored. To improve power of findings, there is need for additional studies with the large sample size. There was no assessment of preconception and early pregnancy dietary intake or maternal baseline 25(OH)D, because these patients were enrolled at the time of diagnosis of preeclampsia. So the impact of these factors on the development of preeclampsia could not be determined. Levels of vitamin D at different gestations associated with both adverse maternal and infant outcomes should be determined in a longitudinal cohort study.

CONCLUSION

At an average of 30 weeks pregnancy, the levels of 25(OH)D in both preeclamptic and normotensive women showed a high deficiency (95%) with no significant difference. An apparent inverse correlation between serum vitamin D and systolic blood pressure and arterial pressure in preeclamptic group in this study although statistically insignificant, may be probably because of small sample size. This needs further study with a larger sample size.

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