DOI: 10.7860/JCDR/2019/40496.12782

Original Article

Obstetrics and Gynaecology Section

Comparison of Vitamin D Levels in Pre-eclamptic and Normotensive Pregnant Women in a Tertiary Care Centre

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ABSTRACT

Introduction: Pre-eclampsia is a multi-system disorder of pregnancy that is significantly contributes to maternal and fetal morbidity and mortality rates. The aetiology of pre-eclampsia remains unknown. Besides other risk factors, attention has been drawn towards vitamin D deficiency as a causative factor for pre-eclampsia.

Aim: To compare vitamin D levels in pre-eclampsia and normotensive pregnant women.

Materials and Methods: A prospective comparative study was conducted in the Department of Obstetrics and Gynaecology, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda, Punjab, India from January 2017 to October 2018. Thirty pregnant women with pre-eclampsia were compared with equal number of normotensive pregnant women for serum vitamin D levels. MS Excel 2010 software was used to analyse the data. Quantitative data were expressed as a mean±standard deviation.

Results: It was observed that vitamin D deficiency was invariably present in all pregnant women but the mean vitamin D level was significantly lower in Pre-eclampsia group as compared to Control group (8.7±5.32 and 14.2±7.88 ng/mL respectively) with $p<0.05$ which was statistically significant.

Conclusion: The present study revealed significant association between maternal serum vitamin D deficiency and occurrence of pre-eclampsia. Vitamin D supplements should be added to all the antenatal patients as routine screening to prevent the risk of pre-eclampsia.

Keywords: Calcium supplementation, Pre-eclampsia, Trophoblast, Vitamin D deficiency

INTRODUCTION

Pre-eclampsia is a disease specific to pregnancy [1-3]. It is a deadly complication of pregnancy, causing around 60,000 maternal deaths annually, out of which 25% cases were of fetal growth restriction and 15% preterm births in most of the countries [4]. Thus, it remains a leading cause of maternal and perinatal morbidity and mortality [5]. It is characterised by new onset of hypertension which presents after 20 weeks of gestation with arterial pressure exceeding 140/90 mmHg on at least two occasions >4 hours apart and proteinuria >300 mg/dL/24 hour, urine albumin >2+ on dipstick testing in a previously normotensive woman [6]. Pathogenesis of pre-eclampsia is complex and yet to be fully determined. It is a two stage disorder. The key features are abnormal trophoblastic invasion of spiral arteries, inappropriate endothelial cell activation and exaggerated inflammatory response [2,3]. The contributing factors are diabetes, chronic hypertension, chronic kidney diseases, nulliparity, twin pregnancy, pre-eclampsia or eclampsia, obesity and various immune disorders. In addition to all these factors, an insufficient diet, especially in terms of calcium, magnesium, selenium and vitamin A and C, also contributes to pre-eclampsia.

Vitamin D is a pro-hormone known to regulate a number of human genes, thus explaining its correlation to the potential risk to various problems like hypertensive disorders, diabetes, cancer, asthma, allergy, autoimmune disorders and depression. It exerts the hormonal action via binding to nuclear vitamin D receptors including placenta and uterine placental bed that is found in the body. The active form of vitamin D, 1,25-dihydroxyvitamin D3, has been drawn to adjust the transcription and function of genes associated with normal implantation, placental invasion, and angiogenesis. It also inhibits release of adhesion molecules from endothelial cells. Maternal vitamin D deficiency may increase the inflammatory reaction. Vitamin D deficiency may also increase the risk of hypertension. This has been postulated that during pregnancy, due to angiogenic and anti-inflammatory effects, Vitamin D plays a role in implantation and placental function. However, it helps in directing immune responses at the fetoplacental interface with immunological adaptation to reduce the risk of inflammation and infection [7]. Hence, the present study was taken up to compare Vitamin D levels in pre-eclamptic and normotensive pregnant women.

MATERIALS AND METHODS

This was a prospective comparative study conducted in the Department of Obstetrics and Gynaecology, AIMSR, Bathinda from January 2017 to October 2018 after taking approval from Research and Ethical Committee (Ref No.: AIMSR/MC/Estt/10/2k/16/3261). Sixty pregnant women attending the outpatient department and labour room, 30 each from normotensive and pre-eclamptic group were included in the study after applying inclusion and exclusion criteria. Inclusion criteria were maternal age of 20-40 years with gestational age of more than 20th weeks of gestation. Whereas, women on vitamin D supplements and women having Diabetes Mellitus (DM), Chronic hypertension, Renal disease, Liver disease, Inflammatory or infective disorders, Severe anaemia, Preterm labor, History of smoking, History of tobacco chewing, Bone diseases, Current steroid therapy, Use of diuretics, beta blockers and Nonsteroidal Anti-Inflammatory Drug (NSAIDs), Multiple pregnancy, Hypoparathyroidism were excluded.

Sample size was calculated using the formula:

$$N=Z^2s^2/d^2$$

Where, Z=Static for 95% confidence interval (=1.96), S=Standard deviation, D=permissible error in mean (5%), Confidence interval=95%

According to a study conducted by, Sadin B et al., the standard deviation of Vitamin D level in pre-eclamptic women is 6.60 [8]. Now using the above formula which uses difference in mean and standard deviation the minimum sample came out to be 27 and

therefore, 30 patients per group were selected for the study. The diagnosis of pre-eclampsia was confirmed with Systolic Blood Pressure (SBP) >140 mmHg and Diastolic Blood Pressure (DBP) >90 mmHg (measured after a period of rest of four hours, twice daily) and proteinuria (>300 mg protein/24 hour) after 20th week of gestation. All the subjects who agreed to participate in the study gave their informed written consent. On enrollment, complete history was taken and clinical examination was done. Total vitamin D levels were measured quantitatively by immune-fluorescence assay test using Vitamin D kit on Tosho AIA 360 fully automatic hormone analyser.

Subjects were classified into four categories according to serum vitamin D levels [9]:

- >20 ng/mL: suboptimal to optimal
- 10-20 ng/mL: mild deficiency
- 5-10 ng/mL: severe deficiency and
- <5 ng/mL: very severe deficiency

STATISTICAL ANALYSIS

MS Excel 2010 software was used to analyse the data. Quantitative data were expressed as a mean±standard deviation. Categorical values were expressed as numbers (n) and percentages (%). Data were analysed with a 95% confidence interval and a p-value <0.05 was accepted as significant. Depending on the type of variables p-value was derived using chi-square and independent sample t-test.

RESULTS

Significant negative correlation was observed between Vitamin D and SBP/DBP in Pre-eclampsia group (p<0.05) while no significant correlation was observed between Vitamin D and SBP/DBP in Control group [Table/Fig-1]. Mean Vitamin D level was significantly lower in Pre-eclampsia group as compared to Control group (8.7±5.32 and 14.2±7.88 ng/mL, respectively) with p<0.05 which was statistically significant [Table/Fig-2]. Vitamin D level <5 ng/mL was associated with a 14.58 fold increase in the odds ratio of pre-eclampsia while Vitamin D level of 5-10 ng/mL was associated with a 11.42 fold increase in the odds ratio of pre-eclampsia [Table/Fig-3].

Parameter	Pre-eclampsia group		Control group	
	r coefficient value	p-value	r coefficient value	p-value
SBP	-0.285	<0.05	0.276	>0.05
DBP	-0.362	<0.05	0.025	>0.05

[Table/Fig-1]: Correlation of Vitamin D levels with systolic blood pressure (SBP)/ diastolic blood pressure (DBP).

Vitamin D (ng/mL)	Pre-eclampsia group		Control group		p-value
	N	%	N	%	
<5	6	20%	2	6.7%	<0.05
5-10	17	56.7%	9	30%	
10-20	5	16.6%	11	36.7%	
>20	2	6.7%	8	26.6%	
Total	30	100%	30	100%	
Mean±SD	8.7±5.32		14.2±7.88		

[Table/Fig-2]: Comparison of Vitamin D levels between the two groups.

Vitamin D (ng/mL)	Pre-eclampsia group		Control group		OR	95% CI	p-value
	N	%	N	%			
<5 ng/mL	6	20%	2	6.7%	14.58	12.16–17.55	<0.05
5-10 ng/mL	17	56.7%	9	30%	11.42	8.26–13.60	<0.05
10-20 ng/mL	5	16.6%	11	36.7%	4.80	2.59–6.09	>0.05
>20 ng/mL	2	6.7%	8	26.6%	1.00	Reference	

[Table/Fig-3]: Adjusted odds ratio for Pre-eclampsia based on Vitamin D levels.

DISCUSSION

Pre-eclampsia of varying degrees of severity adds to number of admissions to the hospitals. In addition to the well known maternal risk factors such as hypertension, diabetes mellitus, antiphospholipid antibody syndrome and obesity, the role of genetic and immunological factors in the pathogenesis of pre-eclampsia has been postulated. The incidence of pre-eclampsia has been found to be lower in summers and higher in winters which suggest a possible role of Vitamin D and sunlight [10]. An inverse association of circulating vitamin D levels with blood pressure has been demonstrated and it has been shown that the treatment with vitamin D supplements reduces blood pressure [10]. Maternal vitamin D deficiency may predispose to a pro-inflammatory response, increased oxidative stress and lead to endothelial dysfunction that characterises pre-eclampsia. In addition, there is evidence showing that vitamin D affects the genes responsible for trophoblast invasion and angiogenesis critical for implantation, which might be an important factor in the pathophysiology of pre-eclampsia [11].

Studies have shown that Vitamin D improves angiogenesis and inhibits release of adhesion molecules from endothelial cells [12,13]. The recommendation of calcium supplementation for individuals with decreased calcium intake to prevent and treat pre-eclampsia by the World Health Organisation (WHO) has increased the popularity of calcium and vitamin D trials in particular thus considering them as "identifiable", "modifiable" risk factors which will in turn, aid in the primary prevention by avoiding occurrence of the disease [14]. Various studies have shown the relationship between pre-eclampsia and vitamin D to be complex. They have shown a low vitamin D level in the second trimester to be an indicator of pre-eclampsia [15-17]. The cut-off level for vitamin D deficiency is acceptable at 20 ng/mL in the latest studies, although previously it was accepted at 15 ng/mL [18,19].

In the present study, serum vitamin D deficiency was universally present in both the groups but it was more severe in the pre-eclamptic group. Sadin B et al., carried out a study to assess the levels of 25(OH)D concentrations in women with pre-eclampsia and healthy women and found that 25(OH)D levels <10 ng/mL were associated with 15 fold increased risk of pre-eclampsia as compared to healthy controls [8]. Dhillon MK et al., also observed lower vitamin D levels in women with pre-eclampsia as compared to healthy women which are consistent with the present study [20]. Hypponen E et al., did a systematic review and meta-analysis and concluded that low maternal serum 25(OH)D levels lead to an increased risk of pre-eclampsia whereas its supplementation lowered this risk [21]. Further supporting the present results, Sahu M et al., evaluated serum vitamin D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester [10]. They found that 75% of the patients in hypertensive group (pre-eclampsia or eclampsia) have very severe vitamin D deficiency (<5 ng/mL) as compared to 25% of normotensive group. The mean serum vitamin D level was 9.06±5.20 ng/mL in pre-eclamptic group compared to 13.67±7.24 ng/mL in healthy pregnant group which was found to be statistically significant.

Bakacak M et al., also compared Vitamin D levels in 40 normotensives, 32 eclamptic and 83 pre-eclamptic pregnant women. The levels were found to be 23.7±5.93, 18.5±5.47 and 19.3±4.31 ng/mL in healthy normotensive women, eclamptic women and pre-eclamptic women, respectively. Thus, Vitamin D levels were found to be significantly lower in the eclamptic and pre-eclamptic groups compared to healthy pregnant women which were statistically significant (p <0.001) [22].

LIMITATION

However, limitations of this study include the stage at which these changes begin to manifest as rising BP with proteinuria is yet to be recognised. Also, the lower limit of Vitamin D levels associated with increased risk of pre-eclampsia is still an area of investigation. Thus a larger study with longer follow-up including neonatal outcome is

required for proper guidelines for serum Vitamin D levels in patients with pre-eclampsia and eclampsia.

CONCLUSION

The present study concludes that Vitamin D levels are lowered in hypertensive women when compared to normotensive pregnant women beyond 20 weeks of pregnancy. Hence, early detection of vitamin D deficiency during pregnancy may be helpful in predicting and preventing the occurrence of hypertensive disorders and its associated co-morbidities thus alleviating maternal morbidity and preterm birth outcomes. Moreover, it may decrease burden on healthcare setup especially in developing countries.

ACKNOWLEDGEMENTS

Authors are immensely grateful to the Department of Obstetrics and Gynaecology, AIMSR, Bathinda for their immense support. Authors also extend their gratitude to the Department of Biochemistry of their institute.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 05, 2018**
Date of Peer Review: **Jan 24, 2019**
Date of Acceptance: **Feb 25, 2019**
Date of Publishing: **Apr 01, 2019**