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A STUDY OF EARLY PREGNANCY VITAMIN D STATUS AND RISK OF PREECLAMPSIA

KEY WORDS: 25-hydroxyvitamin D (25[OH]D) Preeclampsia.

Dr. Arghya Maitra Associate Professor Department Of Obstetrics And Gynaecology Calcutta National Medical College And Hospital Kolkata- 700014

Dr. Priyadarshi Kundu* Rmo Cum Clinical Tutor Department Of Obstetrics And Gynaecology Calcutta National Medical College And Hospital Kolkata- 700014 *Corresponding Author

Dr. Sabyasachi Pujari 3rd Year Pgt Department Of Obstetrics And Gynaecology Calcutta National Medical College And Hospital Kolkata- 700014

Dr. Matiur Hoque 3rd Year Pgt Department Of Obstetrics And Gynaecology Calcutta National Medical College And Hospital Kolkata- 700014

ABSTRACT

AIMS & OBJECTIVE: To examine the association between maternal serum 25-hydroxyvitamin D (25[OH]D) concentration in early pregnancy and the subsequent diagnosis of preeclampsia.

MATERIALS & METHODS: A prospective Cohort study conducted in the Department of Obstetrics and Gynaecology, Calcutta National Medical College, Kolkata, over 100 women attending regular antenatal OPD during the period MARCH 2018 to AUGUST 2019. After inclusion in the present study , blood samples of all the mothers (n=100) were taken to analyze serum levels of Vit-D at 11-14 weeks of gestational age. They were then followed up upto the end of third trimester (term).

RESULTS: In the present study maximum number of patient belonged to 21 – 30 years of age. Association of age vs. preeclampsia was statistically significant (p=0. 0.0007). 78 (78.0%) patients had Vitamin-D deficiency, 14 (14.0%) patients had insufficient Vitamin-D and 8 (8.0%) patients had normal Vitamin-D. In preeclampsia, 4 (66.7%) patients had Vitamin-D Deficiency and 2(33.3%) patient had Insufficient Vitamin-D. Association of Vitamin-D vs. preeclampsia was statistically significant (p=0.0435).

CONCLUSION: Vitamin D deficiency may be an independent risk factor for preeclampsia. Preeclampsia was developed in early age group. Vitamin D supplementation in early pregnancy should be explored for preventing preeclampsia.

INTRODUCTION

Preeclampsia is a multisystem complex disease of pregnancy with grave maternal-fetal and neonatal consequences that complicates up to 8% of pregnancies. Low vitamin D status, among other risk factors, is linked to the development of preeclampsia. Systematic reviews and meta-analyses have concluded that low serum vitamin D levels (25-hydroxyvitamin D [25OHD]) in pregnancy are associated with a higher risk of preeclampsia and suggest a preventive role of vitamin D supplementation. National Institute of Child Health and Development (NICHD) trial started vitamin D supplementation before 20 weeks of gestation. A meta-analysis of the abnormal maternal biomarkers found during the first trimester of pregnancy in women who developed preeclampsia and the findings of previous studies on the association of preeclampsia and low vitamin D status in early pregnancy suggests the necessity of early serum vitamin D level surveillance and modification in pregnancy.

Vitamin D3, also known as calciferol, is a prohormone that plays an important role in calcium homeostasis and bone health in addition to its neuromuscular functions. Several studies reported the relationship between maternal Vitamin D deficiency and adverse maternal and fetal outcomes. In the last two decades, the non-classical function of Vitamin D has been suggested; it regulates a large number of human genes (~200 genes), resulting in a wide range of autocrine effects in different tissues. For example, Vitamin D is involved in regulation of cell proliferation, cell differentiation, and apoptosis. It exerts immune responses through regulation of the innate and adaptive immunity. This explains the correlation of vitamin D deficiency to the potential risk of a series of conditions like hypertensive disorders, diabetes mellitus, cancer, multiple sclerosis, allergy, asthma, autoimmune and infectious diseases as well as depression.

Hypertensive disorders of pregnancy are the most common medical complication of pregnancy and its association with vitamin D deficiency is worth discussing. Hypertensive disorders affect 7 -15% of all gestations and account for potential maternal and perinatal risks and outcome. It includes gestational hypertension, preeclampsia, eclampsia, chronic hypertension, preeclampsia superimposed on chronic hypertension.

In India incidence of preeclampsia is 5-15%, eclampsia is 1 in 500 to 1 in 30. About 16% of maternal deaths were attributed to hypertensive disorders in developed countries and over half of these were preventable. Molecular data confirmed with pathological studies directed towards a two-stage disorder. The key features in the pathogenesis of preeclampsia are abnormal trophoblastic invasion of spiral arteries, inappropriate endothelial cell activation and exaggerated inflammatory response. The known racial disparity in preeclampsia, with black women being more likely to develop severe preeclampsia than white women, suggests that Vitamin D may be relevant.

It has been suggested that preeclampsia and eclampsia results from breakdown of tolerance to the developing fetus after maternal immune maladaptation. Vitamin D is a seco-steroid prohormone which has direct effect on molecular pathways such as trophoblastic invasion and immunomodulation.

During pregnancy, Vitamin D may play a role in implantation and placental function due to angiogenic and anti-inflammatory effects. It is important in directing immune responses at the fetal-placental interface as well as immunological adaptation to reduce the risk of inflammation and infection.

Preeclampsia and eclampsia of varying degrees of severity form a considerable portion of admission in hospitals. Despite the considerable morbidity and mortality, the cause of preeclampsia and eclampsia has remained enigmatic.

METHOD

The study was conducted in the Department of Obstetrics & Gynaecology of Calcutta National Medical College & Hospital, Kolkata during the period of March 2018 to August 2019. This study comprises of 100 women attending regular antenatal OPD were included.

Sampling done based on the following Inclusion and Exclusion criteria:

Inclusion Criteria

1. Informed consent.
2. Pregnant women not received Vitamin D supplementation previously.

Exclusion Criteria

1. Patients not giving consent.
2. Patient with significant medical illness like

- Thyroid, parathyroid or adrenal disease,
- Nephrocalcinosis
- Hepatic or renal failure,
- Metabolic bone diseases
- Those taking medications that might affect vitamin D metabolism e.g.- anti-epileptics, glucocorticosteroids ,.anti-estrogens etc
- Multiple Pregnancies

The present study is a prospective cohort study which aims to determine the association between serum level of Vit-D in early pregnancy and development of subsequent pre-eclampsia in late pregnancy near term.

The study population (n=100) included women in early pregnancy (11-14 weeks of gestation) attending the Antenatal clinic at Calcutta National Medical College from March 2018 to August 2019. In the case of willingness to participate in the study ,they were given specific proforma to fill in and their serum levels of Vit-D were analyzed. Inclusion and Exclusion criteria were satisfied.

After inclusion in the present study , blood samples of all the mothers (n=100) were taken to analyze serum levels of Vit-D at 11-14 weeks of gestational age .They were then followed up upto the end of third trimester(term).

Identification of patients with pre-eclampsia were done through clinical examination and review of laboratory results (e.g.-Blood pressure of 140/90 mm of Hg or higher in sitting position and proteinuria of 2+ or more.

Serum Vit-D levels for finding association with pre-eclampsia are: Deficiency:<20 ng/ml, Insufficiency 20-30ng/ml and Normal is >30ng/ml of blood.

Statistical Analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 25.0; SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. A chi-squared test (2 test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

Explicit expressions that can be used to carry out various *t*-tests are given below. In each case, the formula for a test statistic that either exactly follows or closely approximates a *t*-distribution under the null hypothesis is given. Also, the appropriate degrees of freedom are given in each case. Each of these statistics can be used to carry out either a one-tailed test or a two-tailed test.

Once a *t* value is determined, a *p*-value can be found using a table of values from Student's t-distribution. If the calculated *p*-value is below the threshold chosen for statistical significance (usually the 0.10, the 0.05, or 0.01 level), then the null hypothesis is rejected in favour of the alternative hypothesis.

p-value ≤ 0.05 was considered for statistically significant.

RESULTS

The study population (n=100) included women in early pregnancy (11-14 weeks of gestation) attending the Antenatal clinic at Calcutta National Medical College from March 2018 to August 2019.

In the present study maximum number of patient belonged to 21 – 30 years of age. 4 (4.0%) patients had ≤20 years of age, 87 (87.0%) patients had 21-30 years of age and 3 (9.0%) patients had >30 years of age. The mean age (mean± s.d.) of patients was 24.6300 ± 2.4106 years. In preeclampsia, 4 (66.7%) patients had 21-30 years of age. Association of age vs. preeclampsia was statistically significant (p=0. 0.0007). In preeclampsia, the mean age (mean± s.d.) of patients was 21.6667 ± 2.5033 years. Distribution of mean age vs. preeclampsia was statistically significant (p=0.0113). 9 (9.0%) patients had P0+0, 18 (18.0%) patients had P0+1, 17 (17.0%) patients had P1+0, 20 (20.0%) patients had P1+1, 16 (16.0%) patients had P2+0 and 20 (20.0%) patients had P2+1. In preeclampsia, 3 (50.0%) patients had P0+1, 2(33.3%) patients had P1+1 and 1 (16.7%) patients had P2+0. Association of parity vs. preeclampsia was not statistically significant (p=0.2107). 9 (90.0%) patients had upper class, 40(40.0%) patients had lower middle class, 41(41.0%) patients had middle class and 10(10.0%) patients had lower class. In preeclampsia, 3(50.0%) patients had lower middle class, 1(16.7%) patient had middle class and 2(33.3%) patients had lower class. Association of SES vs. preeclampsia was not statistically significant (p=0.1625).

78 (78.0%) patients had Vitamin-D deficiency, 14 (14.0%) patients had insufficient Vitamin-D and 8(8.0%) patients had normal Vitamin-D. In preeclampsia, 4 (66.7%) patients had Vitamin-D Deficiency and 2(33.3%) patient had Insufficient Vitamin-D. Association of Vitamin-D vs. preeclampsia was statistically significant (p=0.0435). In preeclampsia, the mean Vitamin-D (mean± s.d.) of patients was 16.4667 ± 3.7787. Distribution of mean Vitamin-D vs. preeclampsia was statistically significant (p=0.0597). 71 (71.0%) patients had anemia. In preeclampsia, 1 (16.7%) patient had no Anemia and 5 (83.3%) patients had Anemia. Association of Anemia vs. preeclampsia was statistically significant (p=0.0237599079). 14 (14.0%) patients had diabetes. In preeclampsia, 6(100.0%) patients had Diabetes. Association of Diabetes vs. preeclampsia was not statistically significant (p<0.0001).

4 (4.0%) patients had cardiac disease. In preeclampsia, 4 (66.7%) patients had no Cardiac Disease and 2(33.3%) patients had Cardiac Disease. Association of Cardiac Disease vs. preeclampsia was statistically significant (p=0.0067). 6 (6.0%) patients had respiratory disease. In preeclampsia, 4 (66.7%) patients had no Respiratory Disease and 2(33.3%) patients had Respiratory Disease. Association of Respiratory Disease vs. preeclampsia was statistically significant (p=0.0432522594). 8 (8.0%) patients had HTN. In

preeclampsia, 3 (50.0%) patients had no HTN and 3 (50.0%) patients had HTN. Association of HTN vs. preeclampsia was not statistically significant (p=0.0717182133).

40 (40.0%) patients had previous uterine operation. In preeclampsia, 2 (33.3%) patients had no Previous Uterine Operation and 4 (66.7%) patients had Previous Uterine Operation. Association of Previous Uterine Operation vs. preeclampsia was not statistically significant (p=0.3444200138). 4 (4.0%) patients had Previous IUFD. In preeclampsia, 6 (100.0%) patients had no Previous IUFD. Association of Previous IUFD vs. preeclampsia was not statistically significant (p=0.5763761839). 4 (4.0%) patients had Previous PPH. In preeclampsia, 6(100.0%) patients had no Previous PPH. Association of Previous PPH vs. preeclampsia was not statistically significant (p=0.5763761839).

5(5.0%) patients had Previous Eclampsia 6(6.0%) patients had Preeclampsia. In preeclampsia, 6(100.0%) patients had no Previous Eclampsia. Association of Previous Eclampsia vs. preeclampsia was not statistically significant (p=0.6991965055). The mean BMI (mean± s.d.) of patients was 24.7830 ± 2.0875 kg/m2. In preeclampsia, the mean BMI (mean± s.d.) of patients was 24.1167 ± 1.6018 kg/m2. Distribution of mean BMI vs. preeclampsia was not statistically significant (p=0.4228).The mean GA (mean± s.d.) of patients was 12.3720 ± .8578 weeks. In preeclampsia, the mean GA (mean± s.d.) of patients was 12.2667 ± 1.0482 weeks. Distribution of mean GA vs. preeclampsia was not statistically significant (p=0.7581).

Table 1 : Distribution of Vitamin-D group

Vitamin-D group	Frequency	Percent
Deficiency	78	78.0%
Insufficiency	14	14.0%
Normal	8	8.0%
Total	100	100.0%

Table 2 : Distribution of Previous Eclampsia

Previous Eclampsia	Frequency	Percent
No	95	95.0%
Yes	5	5.0%
Total	100	100.0%

Table 3 : Distribution of Preeclampsia

Preeclampsia	Frequency	Percent
No	94	94.0%
Yes	6	6.0%
Total	100	100.0%

Table 4 : Distribution of Vitamin-D : Preeclampsia

		Number	Mean	SD	Minimum	Maximum	Median	p-value
Vitamin-D	No	94	17.6255	5.2937	12.0000	34.0000	15.7000	0.0597
	Yes	6	16.4667	3.7787	13.6000	21.3000	14.5000	

Table 5 : Distribution of Age in Years : Preeclampsia

	PREECLAMSIA		
Age in Years	**NO**	**YES**	**TOTAL**
≤20 Row % Col %	2 50.0 2.1	2 50.0 33.3	4 100.0 4.0
21-30 Row % Col %	83 95.4 88.3	4 4.6 66.7	87 100.0 87.0
>30 Row % Col %	9 100.0 9.6	0 0.0 0.0	9 100.0 9.0
TOTAL Row % Col %	94 94.0 100.0	6 6.0 100.0	100 100.0 100.0

Table 5 : Distribution of Vitamin-D group : Preeclampsia

	PREECLAMSIA		
Vitamin-D group	**NO PREECLAMPSIA**	**PREECLAMPSIA**	**TOTAL**
Deficiency Row % Col %	74 94.9 78.7	4 5.1 66.7	78 100.0 78.0
Insufficiency Row % Col %	12 85.7 12.8	2 14.3 33.3	14 100.0 14.0
Normal Row % Col %	8 100.0 8.5	0 0.0 0.0	8 100.0 8.0
TOTAL Row % Col %	94 94.0 100.0	6 6.0 100.0	100 100.0 100.0

DISCUSSION

Observational studies evaluating the association between vitamin D and PE have shown inconsistent results and must be interpreted cautiously. This may be a result of issues with study design and methodology, including lack of adjustment of key confounding variables and methods of measuring vitamin D levels.

Most studies controlled for maternal age, body mass index, season, and gestational trimester at sample collection. In addition, some studies also controlled for smoking. Smoking has consistently been shown to reduce the risk of PE and gestational hypertension. This could be due to an association between smoking and lower circulating concentrations of anti-angiogenic proteins and higher concentration of pro-angiogenic proteins. Smoking has also been linked to lower vitamin D concentrations. Smoking may be an important confounder and should be considered in studies linking vitamin D to PE. The pathophysiology of PE may also vary by parity. [1,2,3]

Individuals receive the majority of their vitamin D from sunlight, linking seasonality to the development of PE. Seasonality is also considered a confounder, particularly in studies related to causal effects of vitamin D on PE. Seasonal and latitudinal variation has an effect on vitamin D_3 production in the skin. According to a study in Norway, mothers of children born in August had the lowest risk of PE. [4,5] Risk in this study was highest in the winter months (for December, aOR: 1.26, 95% CI: 1.20–1.31) . Similarly, the incidence PE among white women in the United States was highest in the winter, when production of cutaneous vitamin D_3 is limited in temperate zones and serum 25(OH)D are at their lowest levels. [5] However, despite this known association, not all studies looking at PE as an outcome report information on seasonal, latitudinal variation, sun exposure and lifestyle differences—all of which may differentially expose individuals to sunlight in the sample population.

Maternal dietary intake of vitamin D from foods or supplements may also vary. Oily fish and cod liver oil (n-3 fatty acids) are a rich source of vitamin D. In the Norwegian diet, intake of vitamin D is correlated with intake of long chain n-3 fatty acids. The use of cod liver oil as a food supplement in some diets presents a challenge in determining an isolated effect of vitamin D supplementation. This was noted by Haugen et al., who were unable to control for the intake of fatty acids in their analysis. In their secondary analysis with intake of long chain n-3 fatty acids and vitamin D, a weaker association with the incidence of PE was observed.

We conducted prospective cohort study to determine any

association between serum vitamin D level at early pregnancy(11-14 wks) and development of PE in later stage of pregnancy. Association of PE with different factors are as follows-

AGE :
The mean age if mothers was 24.6300 -+2.4106 years. In pre-eclampsia, 66.7% paients had 21-30 yrs of age. Association of age and pre-eclampsia was found to be significant (p=0.0007).

PARITY ;
In the present study , association between parity and pre-eclampsia was found to be non significant.

BMI:
In this study, the mean BMI of mothers with PE was 24.1167+1.6018 kg/m2. No significant association found.

SOCIO- ECONOMIC STATUS :
No association have been found between socio-economic status of mother and pre-eclampsia. In PE , 50% mothers are from lower middle class , 16.7% mothers are from middle class and 33.3% are from lower class.

ANAEMIA :
In this study , 16.7% mothers had no anaemia and 83.3% had. Association of anaemia with pre-eclampsia was found to be significant (p=0.0237)

In a case-control study done in Kassala hospital ,Sudan-association found between anaemia and pre-eclampsia. The prevalence of preeclampsia and eclampsia was significantly higher in women with severe anaemia (8.2% and 3.3%, respectively) Women with severe anaemia had a 3.6 times higher risk of preeclampsia than women with no anaemia

ASSOCIATED CO-MORBID CONDITIONS :
In this study , no association of pre-eclampsia with any of the co-morbid conditions like Diabetes, Cardiac disease and Respiratory disease was found to be significant.

CHRONIC HYPERTENSION:
In this study 50% of mothers had chronic HTN and 50% mothers had no chronic HTN among the PE .Significant association found between chronic HTN and pre-eclampsia.(0.0017)

PREVIOUS UTERINE OPERATION:
33.3% mothers had previous uterine operations and 66.7% mothers had no previous uterine operation. No association found to be significant between pre-eclampsia and previous uterine operations.

PREVIOUS IUFD :
100% of mothers had no previous IUFD in this study. No statistically significant association found.

PREVIOUS PPH :
100% of mothers had no previous PPH. No association found to be significant.

PREVIOUS ECLAMPSIA :
100% of mothers had no previous eclampsia. No significant association found in this study,

VITAMIN-D STATUS :
In this study, the mean Vitamin –D level of mothers with PE was 17.6255+5.2937. Among pre-eclampsia mothers, 66.7% mothers had Vitamin-D deficiency and 33.3% mothers had Vitamin-D insufficiency. Association between Vitamin-D levels and pre-eclampsia was found to be statistically significant (p=0.0435).

CONCLUSION

In this study we can conclude that:-
Among the study population, 78(78.0%) mothers had Vitamin-D deficiency and 14(14.0%) mothers had Insufficiency of Vitamin-D . Vitamin D deficiency was associated with Pre-eclampsia. Distribution of mean level Vitamin-D was lower in Pre-eclampsia .It was found that most number of Preeclampsia was developed in age group 21-30 years. Preeclampsia was developed in early age group, which was statistically significant. Anemia was found to be more common in pregnant women with Pre-eclampsia. There is also some association between chronic hypertension and Pre-eclampsia.

We concluded that maternal vitamin D deficiency may be an independent risk factor for preeclampsia. Vitamin D supplementation in early pregnancy should be explored for preventing preeclampsia. However, further research work is obligatory to confirm the association of Vitamin-D with development of Pre-eclampsia and role of Vitamin-D supplementation before and during pregnancy.

LIMITATIONS OF THE STUDY

In spite of every sincere effort my study has lacunae.
The notable short comings of this study are:

1. The sample size was very small. Only 100 cases are not sufficient for this kind of study.
2. The study has been done in a single centre.
3. The study was carried out in a tertiary care hospital, so hospital bias cannot be ruled out.
4. Some of the patients possibly on different medications, which were not given by patients for properly during study and evaluation.

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