

Serum Vitamin D Status in Women with Preeclampsia in Ibadan, Nigeria - A Case-Control Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MFA supervised the research work. Author OOS collected the blood samples and managed analyses of all samples. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Preeclampsia is one of the most common complications of pregnancy characterized by high blood pressure, proteinuria and edema. Recent studies suggest that there is a possible link between Vitamin D status and preeclampsia. There is little knowledge on vitamin D status of pregnant and preeclamptic women in this region. This study was carried out to assess serum levels of 25-hydroxy Vitamin D (25(OH)D₃) in normotensive pregnant women and preeclamptic women in Ibadan, Nigeria.

Study Design: Case-Control Study.

Place and Duration of Study: One hundred and twenty women were recruited from Antenatal Clinic of University College Hospital (UCH), Ibadan and Adeoyo Maternity Clinic, Ibadan between January 2016 to August 2017. Sixty normotensive pregnant women (mean age 32.1± 4.8) as

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control and sixty preeclamptic women (mean age 33.4 ± 3.2) as case group. Blood samples were collected at 2nd, 3rd trimester and postpartum.

Methodology: Serum 25-hydroxyvitamin D level was quantified by Enzyme Linked immunosorbent assay (ELISA), and data obtained were analyzed with Student t-test and One way analysis of Variance (ANOVA).

Results: Results showed that preeclamptic women had significantly lower levels ($P < .05$) of Vitamin D at 20 weeks (24.5 ± 4.6 vs 36.59 ± 5.1), 30 weeks (23.8 ± 3.9 vs 34.14 ± 3.7), and postpartum (21.7 ± 5.5 vs 32.62 ± 3.2) when compared to control group. The results of this study show that there is Vitamin D insufficiency in preeclamptic women in the study environment.

Conclusion: Vitamin D insufficiency might be associated with preeclampsia. Vitamin D supplementation and diets rich in Vitamin D, can help to reduce the risk of preeclampsia and also be a possible target for improved pregnancy outcomes in preeclampsia.

Keywords: Preeclampsia; pregnancy; vitamin D; supplementation.

1. INTRODUCTION

Pre-eclampsia is defined as gestational hypertension of at least 140/90 mmHg on two separate occasions ≥ 4 hours apart accompanied by significant proteinuria of at least 300 mg in a 24-hour collection of urine, or a urine dipstick result of 1+ or greater, arising de novo after the 20th week of gestation in a previously normotensive woman and resolving completely by the 6th postpartum week [1]. If left untreated; it progresses to eclampsia, which refers to the development of grand mal seizures in a woman with preeclampsia, in the absence of other neurologic conditions that could account for the seizure. This multisystem disorder affects between 2-8% of all pregnancies worldwide [2]. In Nigeria, the prevalence ranges between 2% to 16.7% [3,4]. Preeclampsia is associated with the following physiologic changes: placental injury, alterations in the interaction between the maternal immune response and the placenta, oxidative stress, imbalance among vasoactive substances, increased production of lipid peroxides, inflammation [5,6]. Women with preeclampsia are often at risk of pulmonary edema, HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low platelets) coagulation defects, cerebral hemorrhage, hepatic or renal failure and ultimately death of both mother and fetus [7].

Vitamin D is a fat soluble vitamin, which occurs in two forms; vitamin D₂ and vitamin D₃. Vitamin D₂ (ergocalciferol) is human-made and added to foods, whereas vitamin D₃ (cholecalciferol) is synthesized in the skin of humans from 7-dehydrocholesterol and is also consumed in the diet via the intake of animal-based foods [8]. Vitamin D, in either the D₂ or D₃ form, is considered biologically inactive until it undergoes

two enzymatic hydroxylation reactions. The first takes place in the liver, mediated by the 25-hydroxylase (most likely cytochrome P450 2R1 [CYP2R1]) which forms 25-hydroxyvitamin D. The second reaction takes place in the kidney, mediated by 1 α -hydroxylase (CYP27B1), which converts 25OHD to the biologically active hormone, calcitriol (1,25-dihydroxyvitamin D), is the major circulating form of vitamin D; it circulates bound to a specific plasma carrier protein, vitamin D binding protein (DBP). DBP also transports vitamin D and calcitriol. The production of vitamin D₃ in skin is dependent on the amount of UVB radiation reaching the dermis and the availability of 7-dehydrocholesterol [9]. Therefore, season of the year, skin pigmentation, latitude, clothing, diet and amount of skin exposed are important factors that can affect serum Vitamin D levels. 25(OH)D circulates bound to the vitamin-D-binding protein, and has a half-life of two weeks, and is an indicator of the endogenous vitamin D status; reflecting the vitamin D produced cutaneously and that obtained from foods or supplements [10]. An adequate 25-OH-D level has been determined to be ≥ 32 ng/ml, vitamin D insufficiency and deficiency are diagnosed at levels of < 32 ng/ml and, < 20 ng/ml 25-OH-D, respectively [9].

Vitamin D plays an important role in bone metabolism through regulation of calcium and phosphate homeostasis and has been reported to aid in gene regulation and expression in early placental development during pregnancy, fetomaternal immune tolerance, and placental antimicrobial and anti-inflammatory responses [11,12]. Data has also supported associations of vitamin D deficiency and preterm birth, decreased birth weight, and hypertensive disease in pregnancy [13,14]. Authors speculate that these conditions may result from the lack of

action of vitamin D in immunosuppression or placental development among deficient patients [13]. Therefore, serum Vit.D insufficiency or deficiency in pregnancy may not only impair maternal and fetal bone health, but also cause various adverse pregnancy outcomes such as preterm birth, fetal intrauterine growth restriction, and preeclampsia. In view of this, it is important to investigate Vitamin D status in Nigerian normotensive and preeclamptic women in their second, third trimester and also three to five days postpartum.

2. MATERIALS AND METHODS

2.1 Study Design

This was a case-control study carried out in the Antenatal Clinic of the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria and Adeoyo Maternity Centre, Yemetu, Ibadan, Nigeria. Ethical Clearance was given by Ethical Committee of The University College Hospital and Oyo State Ethical committee, Nigeria. One hundred and twenty participants were recruited from Antenatal Clinic of the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria and Adeoyo Maternity Centre, Yemetu, Ibadan, Nigeria. The subjects were grouped into two; Group A (Control): Women without high blood pressure ($\leq 120/90$ mmHg) and no proteinuria, singleton uncomplicated pregnancy. Group B: Women clinically diagnosed with high blood pressure ($\geq 140/90$ mmHg) with proteinuria (300 mg protein in 24 hr urine or 2+ dipstick test), with or without edema, and a singleton pregnancy. All subjects were recruited at gestational age of >20 weeks and age range of 18 to 35 years.

2.1.1 Exclusion criteria for cases and control

BMI >30 kg/m², presence of kidney disease, thyroid disorders, diabetes or other chronic disease, history of hypertension, preeclampsia or seizure, anemia and multiple gestation, women on vitamin D supplements.

2.2 Sample Collection

Questionnaires were issued to participants to obtain their demographic and anthropometric data and informed consent form duly signed. 5 mls of blood was collected from the antecubital veins of each of the participants at >20 weeks, 30 weeks gestation and 3 to 5 days postpartum

by using a sterile syringe. The blood was collected into a gel clot separator and centrifuged at 1000 rev./10 mins to get the serum. Serum was stored at -20°C prior to analysis at Institute for advanced Medical Research Training (IMRAT), University of Ibadan, Nigeria.

2.3 Assessment of Vitamin D Status

Vitamin D was assessed by the measurement of serum 25hydroxy vitamin D (25(OH)Vit. D) by the use of Enzyme immunosorbent assay (ELISA) based on the principle of competitive binding. The Calbiotech, Inc. 25 hydroxy Vitamin D Elisa Kit with a sensitivity of 0.67 ng/ml was used for the analysis.

2.4 Statistical Analysis

Data was statistically analyzed by SPSS software version 17. The data were subjected to descriptive analysis, Student's t-test to compare two independent variables and One way analysis of variance (ANOVA) to compare vitamin D levels in second, third trimester and postpartum. Values were expressed as mean $\pm$ Standard deviation of mean and data analyzed at 95% confidence interval. P value $< .05$ was considered statistically significant.

3. RESULTS

In Table 1, the mean age of the normotensive and preeclamptic women was not statistically different. ($P>.05$), while the systolic blood pressure, diastolic blood pressure and BMI of the preeclamptic group were statistically higher than the normotensive group ($P<.05$).

In Table 2, there was a statistically significant decrease in serum Vit.D levels in the preeclamptic group in 2nd, 3rd trimester, and 3-5 days postpartum when compared to the normotensive group.

A statistically significant decrease ($P<.05$) occurred in Vit.D levels in the 3rd trimester when compared to 2nd trimester in normotensive group, and the serum Vit.D levels in the 3rd trimester and postpartum were not statistically different in the normotensive group ($P>.05$).

In the preeclamptic group, the serum Vit.D levels were not statistically different in 2nd, 3rd trimester and postpartum ($P>.05$).

Table 1. Demographic characteristics of control and case group

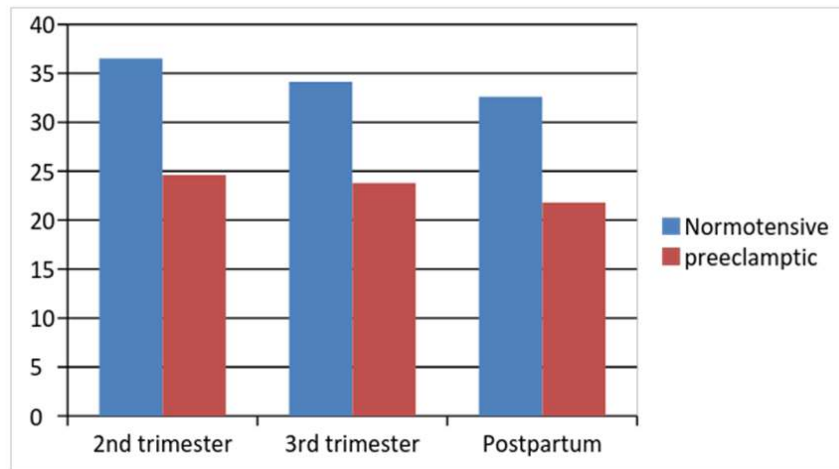
Variables	Group A (Control) n=60 (normotensive)	Group B (Case) n=60 (preeclamptic)	P-Value
Age	32.1 ±4.8	33.4±3.2	0.32
Systolic Blood Pressure	118±17.5	146.8±15.8	0.00*
Diastolic Blood Pressure	71.7 ±7.6	95.9±14.5	0.00*
Body Mass Index(BMI)	23.7± 0.9	30.4±4.6	0.00*

This * indicates that P value is significant at < .05

Table 2. Vitamin D status of normotensive and preeclamptic women at 2nd, third trimester and postpartum

Variables	2 nd trimester (ng/ml)	3 rd trimester (ng/ml)	Postpartum (ng/ml)	P-Value
Group A (Control) n=60 (normotensive)	36.59±5.17 ^a	34.14±3.65 ^b	32.62±3.21 ^b	0.01*
Group B (Case) n=60 (normotensive)	24.5±4.64 ^c	23.8±3.86 ^c	21.7±5.49 ^c	0.16

Values of the same subscript within the same column are not statistically different at ($P>.05$) between the control and case group, while values with different subscripts are significantly different at ($P<.05$)

**Fig. 1. Graphical representation of vitamin D status of normotensive and preeclamptic women at 2nd, third trimester and postpartum**

4. DISCUSSION

In this study, we sought to compare the levels of vitamin D in preeclamptic women and normotensive pregnant women in Nigeria, and the results of the study confirmed that there was vitamin D insufficiency in the preeclamptic group in second, third trimester and postpartum, while the levels of vitamin D in normotensive pregnant women was sufficient in all trimester which was also reported by [15,16,17]. Lower maternal serum vitamin D concentrations in women after diagnosis of preeclampsia has been linked to the pathogenesis of preeclampsia, because of the

influence it has on molecular pathways proposed to be important in the etiology of preeclampsia [18]. A study reported that maternal vitamin D deficiency at less than 22 weeks of gestation was a strong, independent risk factor for preeclampsia, and the author observed a monotonic dose-response relation between maternal serum 25(OH)D and risk of preeclampsia [14].

Vitamin D plays important roles in pregnancy, and therefore its deficiency or insufficiency during pregnancy can be associated with complications of preeclampsia. 1,25-

dihydroxyvitamin D has a direct influence on implantation, placental invasion and angiogenesis [14]. 1,25-dihydroxyvitamin D stimulates the activity of T-regulatory cells, which are vital in supporting placental implantation via immune tolerance [9]. It also participate in modulation of embryo implantation, fetomaternal immune tolerance, and placental antimicrobial and anti-inflammatory responses [11]. Recent studies have also observed that vitamin D can reduce oxidative stress in preeclampsia, promote gene modulation, vascular structure, elasticity and intima-media thickness, and also decrease blood pressure through the renin-angiotensin system (RAS) [19,20].

The low levels of vitamin D metabolism in preeclampsia may be due to reduced placental 1 α -hydroxylase activity [21], resulting in lower circulating calcitriol concentrations in preeclampsia compared to normotensives. In preeclampsia, the metabolism of vitamin D in placental tissue is altered, and these differences may play a role in the abnormal trophoblastic invasion found in these pregnancies [13].

There was a gradual decrease in levels of Vitamin D in 2nd, third and postpartum in the normotensive pregnant women, though the 2nd trimester was not statistically different from the 3rd trimester, the decrease between third trimester and postpartum was significant. This result shows there is a gradual depletion in Vitamin D status as pregnancy progresses which might be due to increased fetal requirement for vitamin D. As the fetus develops, it is entirely dependent on the mother's supply of calcium, and vitamin D is required for normal calcium homeostasis and bone mineralization. It is therefore important that normotensive pregnant women should be on Vitamin D supplementation so as to ensure that the demand by fetus is adequately met and also prevent the risk of developing preeclampsia later in pregnancy.

5. CONCLUSION AND RECOMMENDATION

The role of Vitamin D in pregnancy cannot be overemphasized, and therefore the insufficiency reported in preeclamptic group in Nigeria suggests that vitamin D is implicated in the pathogenesis of preeclampsia. Vitamin D supplementation and diets rich in Vitamin D, can help to reduce the risk of preeclampsia and also be a possible target for improved pregnancy outcomes in preeclampsia.

To prevent gradual depletion of vitamin D as fetus develops in normotensive pregnancies as observed in this study, antenatal supplements, diets rich in vitamin D and adequate exposure to sunlight should be encouraged in antenatal clinics. It is also recommended that women of child bearing age should be well supplemented by taking diets and supplements rich in Vitamin D so as to prevent vitamin D insufficiency later in pregnancy and reduce the risk of developing preeclampsia.

CONSENT

All authors declare that written informed consent was obtained from the patients before sample collection.

ETHICAL APPROVAL

All authors hereby declare that ethical clearance was given by Joint Ethical Committee of the College of Medicine and the University College Hospital, Ibadan Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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