***Original Research Article***

# MATERNAL VITAMIN D STATUS AND RISK OF PREECLAMPSIA IN ABUJA, NIGERIA.


## ABSTRACT

**Aim:** To determine the relationship between maternal serum 25(OH) D concentrations and development of preeclampsia.

**Study design:** A cross sectional comparative study.

**Place and duration of study:** Department of Obstetrics and Gynaecology, University of Abuja Teaching Hospital, Abuja, between March 2016 and February 2017.

**Methodology:** We included 55 women with preeclampsia and 55 healthy women. Data obtained included sociodemographic characteristics, clothing style and duration of exposure to sun light. ELISA method was used for evaluation of serum vitamin D levels.

**Results:** The prevalence of VD deficiency in the population was 15%, while 16.8% and 73% of the participants had insufficient and normal levels respectively. The prevalence of VD deficiency in women with preeclampsia was 20.4% while that in healthy pregnant women was 9.4% (*P*=.19). The mean serum 25-OH-D level of women with pre-eclampsia was significantly lower than that of healthy women (34.5±14.9 vs. 43.5±15.1, *P* = .003). Preeclamptic women with vitamin D insufficiency delivered at a higher gestational age than those with vitamin D deficiency (37.67(2.77) weeks vs. 33.55(2.38) weeks respectively, *P*=.007). In the adjusted analysis of cases with vitamin D defficiency, the odds of developing preeclampsia was not statistically significant [odds ratio (OR) = 3.27, CI = 0.99-10.83, *P*=.05]. However, the odds of developing preeclampsia in women with Vitamin D insufficiency was statistically significant (OR = 3.20, CI = 1.02–10.06, = 0.046).

**Conclusion:** An association between vitamin D deficiency and preeclampsia was not demonstrated in this study. The results however suggest that maternal vitamin D insufficiency in late pregnancy is an independent risk factor for preeclampsia.



*Keywords: Vitamin D, Vitamin D status, preeclampsia, Abuja, Nigeria.*

## 1. INTRODUCTION

In recent years, emerging data have suggested that vitamin D is not only critical for the maintenance of bone health and for calcium and phosphate homeostasis, but also imposes multisystem regulatory effects that modulate overall well-being and health. This is seen in the growing body of literature that suggests that an individual's vitamin D status may adversely impact reproductive functions. Preeclampsia remains a significant contributor to maternal morbidity and mortality[1, 2] and this has made its primary prevention a subject of active research. With regard to this, efforts have been directed on the use of anti-inflammatory agents and micronutrients, including vitamin D.[3]

Vitamin D and its active metabolites are now known to act as mediators of immune function in general and as such, more recently, associations between low vitamin D status and increased risk for various non-skeletal morbidities have been recognized [4, 5, 6]. Studies have suggested that vitamin D deficiency might be a risk factor for preeclampsia[7] and plausible mechanisms include its role in calcium metabolism, increased production of inflammatory cytokines such as TNF-α and decreased function of T-regulatory cells which result in abnormal trophoblastic invasion [7, 8].

Vitamin D deficiency has been found in up to 50% of pregnant women and it has also been noted to have increased incidence among persons of African American race [5]. Other factors that have been suggested to affect vitamin D levels include use of sunscreen, decreased sun exposure, insufficient dietary intake, covering dressing style, effect of northern latitude and seasonal changes[9, 10, 11].

Although results from studies investigating the link between 25(OH) D levels and adverse pregnancy outcomes are contradictory more studies are beginning to reveal that vitamin D deficiency may be risk factor for preeclampsia [5,7,8,12,13,14]. In addition, summaries of evidences[15, 16] concluded that there was a significant relationship between vitamin D deficiency and increased risk of preeclampsia. Studies from sub-Saharan Africa were however scarce.

In areas like ours, socio-cultural practices, deficient diet and lack of supplementation in the public health clinics may significantly impact vitamin D levels in the pregnant population. All these, coupled with the fact that black women are more likely than white women to develop preeclampsia[13] justifies the need to carry out this study. Also, because pre-eclampsia is one of the major causes of maternal morbidity and mortality, establishment of this relationship in our environment is pertinent so that specific measures needed to prevent hypovitaminosis D and its attendant complications can be put in place.

## 2. MATERIAL AND METHODS

### Study Design

The study was a cross sectional comparative study involving 55 preeclamptic and 55 normotensive women attending antenatal clinic or admitted for delivery in the maternity and labour wards of the University of Abuja Teaching Hospital, Abuja.

**Study Location**

University of Abuja Teaching Hospital is a government owned tertiary institution situated in Gwagwalada, a high population density area in Abuja, Nigeria's Federal capital territory. Abuja is located between latitudes 8◦25′and 9◦25′north of the equator and experiences three weather conditions annually. This includes a warm, humid rainy season an intense dry season and a short harmattan season which comes in between. The rainy season begins from April and ends in October, with daytime temperatures reaching 28 °C (82.4 °F) to 30 °C (86.0 °F). In the dry season, daytime temperatures can reach as high as 40 °C (104.0 °F).

**Study subjects**

Cases were women with a diagnosis of preeclampsia in the third trimester and admitted for delivery. Exclusion Criteria included those with pre-gestational hypertension, renal disease, diabetes mellitus, multiple gestation, patients on anticonvulsants, antiretroviral therapy or those with weight greater than 90kg. The control patients were excluded by the same criteria and comprised of normal healthy pregnant women who had given consent. They were matched according to parity (nulliparous vs multiparous) and gestational age (within 1 week).

**Data Collection**

Data was collected with the aid of a proforma and included information on sociodemographic characteristics, parity and gestational age. Information on duration of sun exposure and clothing style to reflect percentage body surface area exposed and was also obtained. Examination and investigations carried out were weight, blood pressure, urinalysis and serum vitamin D levels.

**Preeclampsia** was defined as systolic blood pressure persistently 140 mm Hg or higher and/or diastolic blood pressure persistently 90 mm Hg or higher for the first time after 20 weeks gestation with proteinuria.

**Proteinuria** was defined as a urine sample of 1+ protein or more. 1+ of protein was confirmed by a catheter specimen.

**Sun exposure** was considered adequate when a person is exposed for more than 15min while the type of clothing worn reflected the body surface area exposed. According to the rule of nines, the head and neck sun skin exposure accounts for 9%, each arm for 9%, each leg for 18%, and the front and back torso for 18% each.

**Specimen Collection**

Non fasting blood samples were collected by venipuncture using regular red-top vacutainers. These were allowed to clot and serum separated by centrifugation at room temperature. These were stored at$-20^0$C till time of analysis.

**Laboratory method description**

Serum vitamin D levels was measured with the Calibiotech, Inc. 25-hydroxy (25-OH) Vitamin D enzyme linked immunosorbent assay (ELISA) kit, a solid phase ELISA which is based `on the principle of competitive binding. Microtitre wells coated with anti-Vitamin D antibody were incubated with Vitamin D standards, controls, patient samples (serum), and vitamin D-Biotin conjugate (biotin-labeled vitamin D) at room temperature for 90 minutes. During this incubation, a fixed amount of the biotin-labeled vitamin D competes with the endogenous Vitamin D in the patient's sample, standard, or quality control serum for a fixed number of binding sites on the anti-Vitamin D antibody impregnated wells. A wash step was then undertaken and bound Vitamin D-Biotin is detected with Streptavidin-HRP (Horseradish Peroxidase) by the

addition of the latter. Streptavidin-HRP conjugate immunologically bound to the well progressively decreases as the concentration of Vitamin D in the specimen increases and vice versa. Unbound SA-HRP conjugate was then removed by decanting the contents of the wells and the wells are washed. Next, a solution of Tetramethylbenzidine (TMB) Reagent was added and incubated at room temperature for 30 minutes, leading to the generation of a blue color. The color development was stopped with the addition of a stop solution, and the absorbance measured spectrophotometrically at a wavelength of 450 nm using a microplate reader. The concentration of the standard against the absorbance was plotted, to produce a standard curve and the values of the sample were read off the curve. The color intensity is inversely proportional to the concentration of 25(OH)D in a patient's sample. The assay measured both Vitamin D2 and D3.

The sensitivity of the test kit was 0.67ng/ml while its intra-assay mean according to the guideline of the kit for high and low human standards was 8.1ng/ml with CV = 3.87%, 25.3ng/ml with CV = 6.36% and 35.9ng/ml with CV = 4.62%. The inter-assay mean was 7.9ng/ml with CV = 4.55%, 23.4ng/ml with CV = 6.95% and 37.6ng/ml with CV = 5.38%.

Vitamin D deficiency was defined by levels less than 20 ng/ml, while levels between 20 ng/ml and 29 ng/ml were regarded as insufficient and greater than or equal to 30 ng/ml were normal 25-OH-D concentrations[17, 18].

**Outcome measures**

The primary outcome measure was vitamin D deficiency in both groups while secondary outcome was gestational age at delivery among patients with preeclampsia and vitamin D deficiency.

**Data analyses**

Data analysis was done using Statistical Package for the Social Sciences (spss) version 20 (SPSS Inc., Chicago, IL,USA) software. Continuous variables were presented as mean(SD) while categorical variables were presented as proportions. The analysis of categorical variables was done using the Pearsons chi-square test and Fishers exact test while the independent samples *t*-test and ANOVA test were used for comparing continuous variables between groups. Logistic regression was used to determine the relationship between vitamin D status and risk of preeclampsia after adjusting for potential confounders like age, parity and weight. P value less than .05 was considered statistically significant in all of the analysis.

## 3. RESULTS AND DISCUSSION

**RESULTS**

Out of the 110 samples obtained, 54 cases and 53 controls were successfully analyzed. Three cases were excluded because the results were below the detectable range. Overall, the mean serum 25-OH-D level in the study population was 38.98ng/ml with a range of 14.10ng/ml -83.50ng/ml. Also, the overall prevalence of vitamin D deficiency was 15%, while 16.8% and 68.2% of the participants had insufficient and normal levels respectively.

The characteristics of the study population are shown in Table 1. Women with preeclampsia had significantly higher blood pressures compared to the healthy women, ($P < .001$). In addition to age and body weight, other baseline characteristics like gestational age, educational status, religion and occupation were similar between the two groups.

**Table 1:** Sociodemographic characteristics of study subjects

| | **Pre-eclampsia** | **Healthy women** | |
|---|---|---|---|
| | n=54 | n=53 | |
| **Characteristics** | Mean (SD) or n (%) | Mean (SD) or n (%) | ***P*-value** |
| **Age(yrs)** | 30.0(4.9) | 29.0(4.9) | .27 |
| **Parity** | 2.8(2.1) | 2.2(1.3) | .08 |
| 1st preg | 21(38.9) | 20(37.7) | .90 |
| >1 preg | 33(61.1) | 33(62.3) | .90 |
| **Gestational age (weeks)** | 35.4(3.4) | 35.5(3.5) | .90 |
| **Education** | | | |
| Illiterate | 2(3.7) | 0(0.0) | .495 |
| Primary | 6(11.1) | 3(5.7) | .49 |
| Secondary | 16(29.6) | 13(24.5) | .67 |
| Tertiary | 30(55.6) | 37(69.8) | .16 |
| **Religion** | | | |
| Christian | 41(75.9) | 38(71.7) | .62 |
| Muslim | 13(24.1) | 15(28.3) | .62 |
| **Occupation** | | | |
| civil servant | 18(33.3) | 18(34.0) | .95 |
| Trader | 15(27.8) | 11(20.8) | .40 |
| Artisan | 2(3.7) | 1(1.9) | .57 |

| | | | |
|---|---|---|---|
| House wife | 13(24.1) | 15(28.3) | .62 |
| Unemployed | 1(1.9) | 2(3.8) | .62 |
| Student | 5(9.3) | 6(11.3) | .73 |
| **Weight (kg )** | 73.9(9.9) | 74.5(9.2) | .78 |
| **Systolic blood pressure (mmHg)** | 170.2(16.8) | 108.7(10.0) | <.001 |
| **Diastolic blood pressure (mmHg)** | 107.2(13.8) | 64.5(8.0) | <.001 |

Table 2 compares the vitamin D status of the two groups. The prevalence of vitamin D deficiency among women with preeclampsia was 20.4% while that of healthy women was 9.4%. This however was not statistically significant (*P* = .19). The prevalence of Vitamin D insufficiency was also similar in both groups (22.2% vs. 11.3%, *P* = .21 respectively). Conversely, the proportion of healthy women with normal serum vitamin D was significantly higher than in women with preeclampsia (79.2% vs. 57.4%, *P* = .03).

**Table 2:** Comparison of Vitamin D Status of study subjects

| | **Pre-eclampsia** | **Healthy women** | |
|---|---|---|---|
| | n=54 | n=53 | |
| **Vitamin D status** | n (%) | n (%) | ***P*-value** |
| **Deficient (<20.0 ng/mL)** | 11(20.4) | 5(9.4) | .19 |
| **Insufficient (20.0–29.9 ng/mL)** | 12(22.2) | 6(11.3) | .22 |
| **Normal levels(≥30 ng/mL)** | 31(57.4) | 42(79.2) | .03 |

Table 3 compares serum vitamin D levels and other associated factors in the study subjects. It shows that the mean serum 25-OH-D levels of women with preeclampsia was significantly lower when compared to that of the healthy women (34.5(14.9) vs. 43.5(15.1), *P* = .003). Other factors related to vitamin D status like time of exposure to sunlight and mean body surface area exposed were similar in both groups, *P* = .99 and *P* = .19 respectively. Within the study population, none of the women were taking vitamin D supplements and so this was not included in the table.

**Table 3:** Comparison of serum vitamin D levels and other associated factors in study subjects

| Characteristics | Pre-eclampsia<br>n=54<br>Mean (SD) or n (%) | Healthy women<br>n=53<br>Mean (SD) or n (%) | *P*-value |
|---|---|---|---|
| **Serum Vit D(ng/ml)** | 34.5(14.9) | 43.5(15.1) | .003 |
| **Sufficient exposure to sunlight**<br><15 mins day$^{-1}$ | 2(3.7) | 2(3.8) | .99 |
| >15 mins day$^{-1}$ | 52(96.3) | 51(96.2) | |
| **%Body surface area exposed** | 30.1(12.0) | 27.1(11.3) | .19 |

Within the categories of vitamin status of women with preeclampsia, there was a statistically significant difference in gestational age (GA) at delivery as determined by one-way ANOVA ((*F* (2,51) = 5.153, *P* = .009). A Tukey post hoc test revealed that women with vitamin D insufficiency delivered at a statistically significant mean higher gestational age than those with vitamin D deficiency (37.67(2.77) weeks vs. 33.55(2.38) weeks respectively, *P* = .007). There was no statistical significant difference in GA at delivery between the deficient and normal groups (*P* = .85). Other characteristics like age, parity, weight and body surface area exposed were similar within these groups (Table 4).

**Table 4.** Comparison of maternal characteristics by vitamin D status in pregnant women with preeclampsia

| Characteristics | 25(OH)D Deficiency (<20.0 ng/mL) n=11 | 25(OH)D Insufficiency (20.0–29.9 ng/mL) n=12 | 25(OH)D Normal (≥30 ng/mL) n=31 | p-value |
|---|---|---|---|---|
| **Age(yrs)** | 30.1(5.4) | 30.7(4.7) | 29.7(4.9) | .86 |
| **Parity** | 2.6(2.5) | 2.3(1.8) | 3.0(2.0) | .53 |
| **Gestational age at delivery (weeks)** | 33.6(2.4) | 37.7(2.8) | 35.2(3.4) | .009 |
| **Weight (kg )** | 72.6(6.5) | 72.5(10.7) | 74.9(10.8) | .82 |
| **%BSA exposed** | 31.1(11.9) | 28.2(14.43) | 30.5(11.5) | .31 |

Following adjustment for age, parity and body weight in a bivariate logistic regression analysis, maternal vitamin D deficiency was not associated with the development of preeclampsia [odds ratio (OR) = 3.27, CI = 0.99-10.83, *P* = .05]. On the other hand however, women with vitamin D insufficiency had about a three-fold odd of developing preeclampsia and this was statistically significant (OR = 3.20, CI = 1.02–10.06, *P* = .046) (Table 5). Age, parity and weight were not significantly associated with the development of preeclampsia in this study.

**Table 5:** Unadjusted and adjusted ORs for Preeclampsia according to vitamin D status

| Serum 25(OH)D level (ng/ml) | Pre-eclampsia<br>n=54<br>n (%) | Healthy women<br>n=53<br>n (%) | Unadjusted OR(CI) | p-value | Adjusted[a] OR(CI) | *P*-value |
|---|---|---|---|---|---|---|
| **Deficient <20** | 11(20.4) | 5(9.4) | 2.98 (0.94-9.46) | 0.064 | 3.27 (0.99-10.83) | .05 |
| **Insufficient 20–29.9** | 12(22.2) | 6(11.3) | 2.71 (0.92-8.01) | 0.072 | 3.20 (1.02-10.06) | .046 |

[a]*Adjusted for Age, Parity, Weight*

## Discussion

In this study, vitamin D deficiency was defined by a 25-hydroxy vitamin D level of less than 20 ng per milliliter (50 nmol per liter) [18] and using this set point, a prevalence rate of 15% was recorded. This value is low compared to reports of vitamin D deficiency among pregnant women in literature and one possible explanation for this could be that different cut off points have been used by various researchers. Rates of 42%, 61%, 80% and 60–84% have therefore been reported in northern India[19], New Zealand[20], Iran[21] and Netherlands[22] using a threshold of 10ng/ml while rates of about 69-80% have been reported in black pregnant women in studies done in the US[23] and Ethiopia[24] using higher cut off points.

It has been suggested that geographic latitude and cloud cover are among significant factors that affect ultraviolet radiation and vitamin D synthesis in the skin[17]. This may explain the comparatively low prevalence rate recorded in this study, and this is despite the total lack of multivitamin use among the participants. Another factor that may have contributed to this result was their adequate exposure to sunshine which is in abundance in the area the study was undertaken i.e Abuja. Unfortunately, data from similar geographical region and population to help support our findings appear to be sparse and the conflicting result obtained in Ethiopia[24] could be attributed to their clothing habit because the study was carried out in a population which practiced purdah.

With regard to the factors determining amount of serum vitamin D, it seems that effect of latitude, sun exposure and dietary supplementation all have a role to play. This suggestion may seem plausible because the mean serum level of vitamin D in our study was similar to that of the study done in North Carolina [8] (34.5ng /ml vs. 30 ng/ml). The study in northern Carolina was conducted in a higher altitude area but reported regular intake of supplements while our study was conducted in a low altitude region, among women who did not take supplements but were adequately exposed to sunshine. On the other hand, a study which was conducted in the middle-east[25] , where about two thirds of the population

had insufficient sun exposure and also reported low supplement intake recorded significantly lower serum vitamin D levels (18.1ng/ml) among women with preeclampsia. This highlights the role of vitamin D supplementation, and to further support this are the results of a systematic review and metaanalysis on Vitamin D and development of preeclampsia. The report concluded that low maternal serum 25(OH) D concentrations increased pre-eclampsia risk and that vitamin D supplementation lowers this risk [16].

Another major finding of this study was that women who developed preeclampsia had significantly lower maternal serum 25(OH) D compared with healthy women with uncomplicated pregnancies. This is despite suggestions that since the amount of vitamin D is dependent on sunlight exposure, diet and vitamin D supplementation, there are no significant changes in the level of serum vitamin D during pregnancy[7]. Results of some other studies also confirm this[7,8,12,25] and thus, buttress the important role of vitamin D in the etiology of preeclampsia.

In order to determine the relationship between vitamin D deficiency and preeclampsia at the point of delivery, the women were categorized based on their serum vitamin D levels. Our findings revealed that there was no relationship between vitamin D deficiency and preeclampsia. However, vitamin D insufficiency was found to be a risk factor for preeclampsia. This result shows a reversal of the overall findings of similar studies with similar prevalence rates [7,8,12,25], and reasons for this may not be so clear. The study by Abedi et al[7] in Iran had a similar sample size with this study and also had samples taken at delivery but pertinent differences with this study are the fact that it was done in an area of greater latitude and in a population where less than half of the women had sufficient exposure to sunshine. These differences seem to be reflected in their mean serum vitamin D levels which were much lower than what was obtained in this study (17.48 vs. 34.5 ng/ml respectively). Similar differences were also noted in the study by Bodner et al.[12] who also recorded a mean level of 18.16ng/ml. Differences may also have been due to different cut off points used for defining vitamin D deficiency in these studies. Our results however were similar to that obtained in a few other studies[13,26,27]. Take for instance, the study done by Ringrose et al.[13] did not find any relationship between serum vitamin D status and preeclampsia even though the study was done in a high latitude location and third trimester samples obtained. Their serum vitamin D levels were also lower than that obtained in this study (24.68 vs. 34.5ng/ml). Overall results can therefore be said to vary and reason for this may likely be linked to prevailing factors earlier described. There is therefore the need for more studies to be carried out in populations with similar socio-demographic and geographic characteristics like ours so that we will have more data to compare with.

This study showed that preeclamptic women who had vitamin D deficiency delivered at a lower gestational age than those who were not. This result may be explained by reports of increased production of inflammatory cytokines in pregnancies complicated by vitamin D deficiency thereby suggesting a possible role for vitamin D in preventing spontaneous preterm birth through anti-inflammatory and immunomodulatory effects[28]. Baker et al.[29] tested but failed to prove this hypothesis, but this may have been due to the fact that they had used first trimester samples. There were no differences in other maternal characteristics demonstrated between preeclamptic women with and without vitamin D deficiency in this study and this may have been due to the fact that the two groups were matched by parity and gestational age and women

above 90kg were also excluded. Studies that did not match their groups were able to establish significant relationship between serum vitamin D levels and BMI[7, 13], age[7,25] and socioecomomic status[25].

## 4. CONCLUSION

Our study showed that maternal vitamin D deficiency in the third trimester was not associated with preeclampsia. The results however suggest that maternal vitamin D insufficiency in late pregnancy is an independent risk factor for preeclampsia. I recommend that further large, well designed studies and trials be carried out in our environment so as to further corroborate these results. In addition, following results of meta-analysis of studies on vitamin D supplementation alongside recommendations by the Royal college of Obstetricians and Gynaecologists (RCOG)[30], Vitamin D supplementation in pregnancy could be considered as a safe and effective means of preventing preeclampsia.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethical approval was obtained from the University of Abuja Teaching Hospital ethical board (Approval no. UAH/HREC/480).

## REFERENCES


1. World Health Organisation. WHO recommendations for Prevention and treatment of pre-eclampsia and eclampsia, *World Health Organization,* Geneva, Switzerland, 2011. Available at http://whqlibdoc.who.int/publications/2011/9789241548335_eng: (Accessed 27/03/2015)
2. World Health Organisation. Make every mother and child count. In: *The world health report*, World Health Organization, Geneva, Switzerland 2005. Available at http://www.who.int/whr/whr_2005_eng. (Accessed-2/04/2015)
3. Royal College of Obstetricians and Gynaecologists (RCOG). The management of hypertensive disorders during pregnancy. NICE Clinical Guideline No107. London: RCOG Press; 2010.
4. Szabo A. Skeletal and extra-skeletal consequences of vitamin D deficiency. *Orv Hetil.* 2011; 152(33):1312-9.
5. Robinson CJ, Wagner CL, Hollis BW, Baatz JE, Johnson DD. Maternal vitamin D and fetal growth in early-onset severe preeclampsia. *Am J Obstet Gynecol.* 2011; 204 (6):556–4.

6. Hamilton SA, McNeil R, Hollis BW, Davis DJ, Winkler J, Cook C, et al. Profound vitamin D deficiency in a diverse group of women during pregnancy living in a sun-rich environment at latitude 32°N. *Int J Endocrinol.*2010; 2010:917428.
7. Abedi P, Mohaghegh P, Afshary P, Latifi M. The relationship of serum vitamin D with pre-eclampsia in the Iranian women. *Matern Child Nutri.* 2014; 10: 206–212.
8. Baker AM, Haeri S, Camargo CA Jr, Espinola JA, Stuebe AM.. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. *J Clin Endocrinol Metab.* 2010; 95:5105-*9.*
9. Newhook LA, Sloka S, Grant M, Randell E, Kovacs CS, Twells LK. Vitamin D insufficiency common in newborns, children and pregnant women living in Newfoundland and Labrador, Canada. *Matern Bhild Nutr*. 2009; 5(2):186-91.
10. Parlak M, Kalay S, Kalay Z, Kirecci A, Guney O, Koklu E. Severe vitamin D deficiency among pregnant women and their newborns in Turkey. *J Matern Fetal Neonatal Med*. 2014; 30:1-4.
11. Yu CK, Ertl R, Skyfta E, Akolekar R, Nicolaides KH. Maternal serum vitamin D levels at 11-13 weeks of gestation in preeclampsia. *Hum Hypertens* 2013; 27:115-8.
12. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab.*2007;92:3517–3522.
13. Ringrose JS, PausJenssen AM, Wilson M, Blanco L, Ward H, Wilson IW. Vitamin D and hypertension in pregnancy. *Clin Invest Med* 2011; 34 (3): E147-E154.
14. Wei SQ, Audibert F, Hidiroglou N, Sarafin K, Julien P, Wu Y, et al. Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. *BJOG* 2012; 119:832-9.
15. Tabesh M, Salehi-Abargouei A and Esmaillzadeh A: Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis. *J Clin Endocrinol Metabol* 2013; 98:3165-3173.
16. Hyppönen E., Cavadino A., Williams D., Fraser A., Vereczkey A., Fraser W.D. et al. Vitamin D and Pre-Eclampsia: Original data, systematic review and meta-analysis. *Ann. Nutr. Metab*. 2013; 63:331–340.
17. Holick MF. Vitamin D Deficiency. *N Engl J Med*. 2007; 357:266-28.
18. Vitamin D: screening and supplementation during pregnancy. Committee Opinion No. 495. American College of Obstetricians and Gynecologists. *Obstet Gynecol.* 2011; 118:197–8.
19. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *Am J Clin Nutr*. 2005; 81(5):1060–1064.
20. Judkins A, Eagleton C. Vitamin D deficiency in pregnant New Zealand women. *N Z Med J.* 2006 ;119(1241):U2144.
21. Bassir M, Laborie S, Lapillonne A,Claris O, Chappuis MC, Salle BL. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. *Acta Paediatr* 2001; 90577–579.
22. Van Der Meer IM, Karamali NS, Boeke AJP, Lips P, Middelkoop BJ, Verhoeven I et al. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands*. Am J Clin Nutr.* 2006; 84:350–353.
23. Ginde AA, Sullivan AF, Mansbach JM, Camargo CA., Jr. Vitamin D insufficiency in pregnant and non-pregnant women of childbearing age in the United States. *Am J Obstet Gynecol.*2010; 202(5):436–438.
24. Feleke Y, Abdulkadir J, Mshana R, Mekbib T, Brunvand L Berg JP, et al. Low levels of serum calcidiol in an African population compared to a North European population. *Eur J Endocrinol.* 1999;141:358–360

25. Bener A, Al-Hamaq AO, Saleh NM. Association between vitamin D insufficiency and adverse pregnancy outcome: Global comparisons. *Int J Womens Health*. 2013; 5:523–31.

*26.* Schneuer FJ, Roberts CL, Guilbert C, Simpson JM, Algert CS, Khambalia AZ. Effects of maternal serum 25-hydroxyvitamin D concentrations in the first trimester on subsequent pregnancy outcomes in an Australian population. *Am J Clin Nutr* .2014; 99(2):287-295.

27. Shand AW, Nassar N, Von Dadelszen P, Innis SM, Green TJ. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. *BJOG.*2010; 117:1593–1598.

28. Luk J, Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. *Hum Reprod*. 2012;27:3015–27.

29. Baker AM, Haeri S, Camargo CA, Stuebe, A. M., Boggess, K. A. A Nested Case-Control Study of First-Trimester Maternal Vitamin D Status and Risk for Spontaneous Preterm Birth. *Am J Perinatol.* 2011; *28*(9):667–672.

30. Royal College of Obstetricians and Gynaecologists (RCOG). Vitamin D in Pregnancy, Scientific Impact Paper No. 43; 2014.