Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 2| Issue 3 |JULY-SEPT |2012|144-150

Research Article
Biological Sciences

BENEFITS OF VITAMIN D SUPPLEMENTATION IN PREGNANCY FOR PREVENTION OF PREECLAMPSIA

*Parul Singla[1], Anuj Anand Parkash[1], Harbans Lal[1], Smiti Nanda[2]

[1]Dept. of Biochemistry, Pt. B.D. Sharma PGIMS, Rohtak, HARYANA -124001
[2]Dept. of Obstetrics and Gynaecology, Pt. B.D. Sharma PGIMS, Rohtak, HARYANA -124001
*Corresponding Author Email: drparul1@yahoo.co.in

ABSTRACT

Aim: *The role of vitamin D has been studied in growth and reproduction, carcinomas, psoriasis etc. Even in preeclampsia aberration in calcium homeostasis was found to be associated with decreased calcitriol levels. In present study, we investigated the role of vitamin D supplementation in regulation of calcium homeostasis and prevention of preeclampsia.*

Methods: *The study comprised of 200 primigravidae, out of which 100 were managed with routine antenatal protocol (control group) while other 100 women received supplemental vitamin D (60,000 IU, every fortnight) along with routine antenatal protocol (study group) from 28±1 week till 36±1 week of gestation. Serum and urinary parameters of calcium homeostasis were estimated in both the groups and "Odds Ratio" was calculated to know the relative risk of preeclampsia.*

Results: *The incidence of preeclampsia was found to be 10% in Control group and 4% in the Study group. The Odds Ratio for Preeclampsia was found to be 0.375 (95% confidence interval - 0.114-1.24) in Study group. Significant increase in serum and urinary calcium level ($p<0.001$) and decrease in serum phosphorus level ($p<0.01$) was observed in study group at 36 week. The urinary calcium/creatinine ratio was found to be significantly increased in study group ($p<0.001$) and a negative correlation was observed between blood pressure and urinary calcium/creatinine ratio in study group at 36 week.*

Conclusion: *It is suggested that vitamin D supplementation during the third trimester of pregnancy, is efficacious in reducing the risk of preeclampsia by increasing therapeutic effectiveness of calcium supplementation in pregnant women.*

KEYWORDS

Calcium homeostasis, Preeclampsia, Pregnancy, Vitamin D

INTRODUCTION

Preeclampsia is a leading cause of maternal mortality throughout the world and is associated with perinatal morbidity and mortality. It is defined as the development of hypertension (blood pressure over 140/90) and proteinuria during the second half of gestation and it complicates 3-7% of normal pregnancies[1].It has been suggested that vitamin D plays a role in blood pressure regulation. Receptors for 1, 25-dihydroxyvitamin D (calcitriol) have been found in target tissues that regulate blood pressure[2]. There are also indications that both hypocalcemia and hypercalcemia affect blood pressure and in both cases, vitamin D metabolism is involved[3].

It has been observed that decreased calcitriol levels during preeclampsia impair intestinal calcium absorption leading to hypocalcemia. As a compensatory mechanism there is increase in tubular reabsorption of calcium and it leads to hypocalciuria during preeclampsia[4]. It has been reported that hypocalciuria predicts preeclampsia long before clinical manifestation exist[5]. 24- hour urinary calcium excretion and calcium-creatinine ratio are reliable screening parameter of preeclampsia[6]. Further, it has been observed that calcium supplementation alone during pregnancy does not prevent preeclampsia but a combined therapy of calcium and vitamin D_3 has been found to be more efficacious[7].

A tendency towards a lower level of 1, 25 dihydroxy Vitamin D has been reported in preeclampsia[8]. The low level of active vitamin D is due to the reduced amount of 1-alpha hydroxylase. This enzyme is located in kidney and placenta and both of which are known to be affected in Pregnancy Induced Hypertension (PIH) [9]. The pathogenesis of preeclampsia also involves other biological processes that may be directly or indirectly affected by vitamin D, including immune dysfunction, placental implantation, abnormal angiogenesis, excessive inflammation, and hypertension[10]. So, Vitamin D has been hypothesized to influence preeclampsia risk. The search for an effective preventive therapy has therefore been a major focus of obstetrical investigation.

Thus, the present study was undertaken to assess the role of vitamin D supplementation in prevention of preeclampsia.

MATERIALS AND METHODS

This was longitudinal prospective, case controlled study conducted in the Department of Biochemistry, in collaboration with the Department of Obstetrics and Gynaecology, Pt. B.D. Sharma PGIMS, Rohtak. Two hundred primigravidae attending antenatal clinic of the Department of Obstetrics and Gynecology were selected for the study after a written and informed consent as per Declaration of Helsinki[11]. The study was approved by the Institutional Ethical Committee.

The selection criteria included women with singleton pregnancy, below 35 years of age, 28±1 weeks of gestation with normal blood pressure. Women with multiple pregnancy, essential and chronic hypertension, preexisting renal disease, diabetes mellitus and elderly primigravida (age >35 years) were excluded from the study. The women fulfilling the required criteria were randomly divided into two groups without any selection bias.

One hundred pregnant women selected in the control group were managed with the conventional antenatal care (supplementation of calcium, 1000 mg and iron, 40 mg from second trimester of pregnancy) and another one hundred pregnant women in the study group, were supplemented with vitamin D along with conventional antenatal care. Vitamin D was given as oral supplementation (in the form of sachets dissolved in milk), in doses of 60,000 IU once in every two weeks, orally, after 28 week up to 36 week[12].

All the women were thoroughly examined; blood and 24 hour urine were collected. Samples were collected at the time of enrollment (28 ± 1 weeks) and at 36 ± 1 weeks of gestation. All precautions were followed as per Clinical Laboratory Standard Institute (CLSI) guidelines[13]. Blood sample was collected in commercially available plain vial. Sample was allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and analysed immediately. 24 hour urine sample was collected in a sterile container on the same day. Urine was acidified with 6N Hydrochloric acid before measurement of urinary calcium. Presence of albuminuria was assessed by a semiquantitative method using albustix (Siemens health care).

During the follow up period (28th to 36th week), subjects were followed up for development of any signs of preeclampsia (increase in blood pressure, albuminuria, excessive weight gain during routine antenatal visits). Blood samples collected were used for estimation of serum calcium and phosphorus. 24 hour Urine was evaluated for urinary calcium and creatinine and urinary calcium to creatinine ratio was calculated to assess the role of kidneys in regulation of calcium homeostasis in Pregnancy Induced Hypertension (PIH).

All serum and urinary parameters were performed on fully automated Random Access

Analyzer (KONELAB 30, Thermo scientific). Serum and urinary calcium were measured using Arsenazo III method using commercial kits from Randox Laboratories. Serum phosphorus was measured by using commercial kits from Siemens Diagnostics. 24 hour Urinary creatinine was measured by Jaffe's reaction.

The levels of serum and urinary biochemical parameters were compared between the study and control group by unpaired *t* test. For paired samples, Wilcoxon-signed rank test was used. The observed values for these parameters of calcium homeostasis were correlated to the maternal sign of preeclampsia i.e. blood pressure using Pearson's correlation coefficient. $P < 0.05$ was considered significant. The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) analytical software, version 18^{+}.

RESULTS

Mean age group of the women selected in the control group was 19.75 ± 0.52 years and 20.35 ± 0.76 years in the study group. This difference in age was not statistically significant, indicating that the women were age matched. All the antenatal females selected were normotensive at 28 ±1 weeks.

At 36 weeks, ten subjects were found to have blood pressure >140/90mmHg in the control group, whereas only four subjects in study group had blood pressure > 140/90 mm Hg. Mean systolic blood pressure in the control group at 36 ±1 week was 129.1±0.88mmHg and at 28±1 week was 121.9±0.92mmHg. This rise in mean systolic blood pressure in the control group was statistically significant. The mean systolic blood pressure in the study group was 120.2 ± 0.83 mmHg at 28±1 weeks and 122 ± 0.73 mmHg at 36±1 weeks and the difference was not found to be significant (p>0.05). Mean diastolic blood pressure remained unaltered at gestation age of 36 weeks with or without vitamin D supplementation (**Table 1**).

Table 1: Comparison of Blood Pressure between the two groups at 28 and 36 weeks

Parameter	Control group (n=100) Mean ±SE		Study group (n=100) Mean ± SE	
	28 week	**36 week**	**28 week**	**36 week**
Systolic BP(mmHg)	121.9 ± 0.92	129.1 ± 0.88*	120.2 ± 0.83	122.0 ± 0.73**
Diastolic BP (mmHg)	78.1 ± 0.69	80.2 ± 0.73	77.4 ± 0.55	78.7 ± 0.55

*** p value < 0.001 with the corresponding group at 28 week**
****p value < 0.001 when compared with the control group at 36 week**

At 28±1 weeks, albuminuria was nil/trace in both groups. At 36 ± 1 weeks, albuminuria was present in the "++" range (~ 100mg/dl) in seven primigravidae and "+++"(~ 300 mg/dl) in three primigravidae in the control group. In the study group albuminuria was "++" in four primigravidae who also demonstrated the presence of high blood pressure at 36±1 week. Thus, it has been observed that the incidence of preeclampsia was 10% in the control group at 36±1 week and 4% in vitamin D supplemented group. The relative risk for preeclampsia development was found to be 0.375 (95% confidence interval= 0.114-1.24) in study group and 2.67(95% CI 3.89 - 1.67) in control group.

A comparative analysis of 200 subjects in control and study group for serum and urinary biochemical parameters at 28 & 36 week are shown in Table 2 with significant p values.

Table 2: Comparison of Biochemical Parameters in two groups at 28 and 36 week

Parameter	Control Group (n=100) Mean ±SE		Study group (n=100) Mean ±SE	
	At 28 week	At 36 week	At 28 week	At 36 week
Serum calcium(mg/dl)	8.75±0.065	8.6±0.056*	8.57±0.031	9.33±0.056*,**
Serum Phosphorus(mg/dl)	3.51±0.46	3.63±0.41	3.57±0.30	3.06±0.48*,**
24 hr Urinary Calcium(mg/day)	147.7±4.15	138.9±4.03#	147.8±3.20	168±3.43#,**
24 hr Urinary Creatinine(mg/day)	1465.2±2.23	1501.7±2.38#	1481.9±2.28	1484±2.01
Calcium/Creatinine ratio	0.1±0.001	0.09±0.009#	0.09±0.009	0.12±0.012#,**

*p value < 0.05, # p value <0.01 with the corresponding group at 28 week
** p value < 0.001 when compared with the control group at 36 week

At 28±1 week there is no significant difference observed in serum and urinary parameters. Rise in mean serum calcium level and decrease in serum phosphorus in study group after vitamin D supplementation was statistically significant within the corresponding group ($p<0.05$) and also in comparison with the control group at 36±1 wk ($p<0.001$). Increased serum calcium along with decrease in serum phosphorus resulted into same calcium phosphorus product during the entire study period. A significant negative correlation was observed between serum calcium and blood pressure. When vitamin D was added to the routine protocol, rise in mean serum calcium level with a non-significant change in blood pressure was seen at 36 ± 1 weeks. ($r=-0.214$, $p<0.05$).

In the study group, a significant rise in mean 24hr urinary calcium was seen when compared within the corresponding group ($p<.01$) and in comparison to the control group at 36 ± 1 week (<0.001). In control group, decrease in 24 hr urinary calcium was observed at 36 week in comparison to the mean value of 24 hour urinary calcium at 28 ± 1 week(<0.001). An increase in 24hr urinary creatinine was seen in control group at 36 ± 1 weeks in comparison to the study group but this difference was not significant statistically.

Significantly high urinary calcium to creatinine ratio was observed in study group as compared to control group at 36th week of gestation. When compared within the groups, a significant decrease in ratio was observed with increase in gestation in the control group ($p<0.001$) and it was found to be negatively correlated with blood pressure ($r=-0.677$, $p<0.001$). In the study group, observed ratio increased as gestation period advanced, whereas blood pressure remained unaffected or decreased. This inverse relationship of urinary calcium to creatinine ratio with blood pressure after supplementation of vitamin D was statistically significant ($r=-0.342$, $p<0.001$).

DISCUSSION

Epidemiological studies have reported high incidence of preeclampsia in antenatal women having disturbed calcium homeostasis[14]. Since Vitamin D is one of the hormones involved in regulation of calcium, the present study evaluated the effect of vitamin D supplementation on calcium homeostasis after 28 week of gestation and its effect on blood pressure and calcium homeostasis in pregnancy at 36 weeks.

In the present study, all women were normotensive at 28 weeks. However, at 36 weeks high blood pressure was found to complicate 4% of pregnancies in Vitamin D supplemented group in comparison to 10% in the control group. These findings are in

accordance with Ito et al, who reported the incidence of Pregnancy Induced Hypertension (PIH) 10.9% when vitamin D3 was supplemented with calcium in comparison to the incidence of 16.9% found in the control group. It has been suggested that vitamin D increases the therapeutic effectiveness of calcium supplementation and combination of vitamin D and calcium will be more efficacious in reducing the incidence of PIH than calcium alone[7]. The relationship between blood pressure and vitamin D supplementation in pregnancy has not been studied extensively. However, a study carried out by pfeifer et al [15], concluded that combined oral supplementation of vitamin D and calcium lowered the systolic blood pressure in hypertensive women. Also, in various hypertensive rat models oral vitamin D supplementation helped in reducing blood pressure and also inhibited rennin expression[16-17]. Kaur et al, in another study found that supplementation of vitamin D in 60,000 IU during 6th and 7th month of pregnancy increases protein, DNA and RNA content of placenta and improved the placental growth[18]. It has been known that placenta expresses 1- alpha hydroxylase, required in vitamin D metabolism. A relative risk of 0.375 with CI of 0.114-1.24 has been observed in the study group. In another study a 27% reduction in risk of pre-eclampsia has been observed after vitamin D supplementation in nulliparous women with odds ratio of 0.73 (95% confidence interval = 0.58-0.92) [19].

In the present study, the incidence and severity of albuminuria at 36±1 weeks, was higher in control group (10%) as compared to the vitamin D supplemented primigravidae (4%). Various recent studies have demonstrated a negative association between albuminuria and Vitamin D levels[20, 21]. Though Vitamin D levels were not analysed in the present study, nonetheless, it can be assumed that Vitamin D supplementation in the study group probably resulted in lower incidence and severity of albuminuria in this group. Recent studies have also demonstrated the reno-protective effects of Vitamin D analogs[22].

In the vitamin D supplemented group a significant increase in serum calcium and a significant decrease in serum phosphate was observed at 36 ± 1 week. These changes can be attributed to the known effects of vitamin D on kidneys and intestine leading to increased calcium levels and decreased phosphate levels. 1,25 (OH)2 vitamin D acts on the intestine and increases the absorption of calcium by inducing the synthesis of protein calbindin 9K-D and increases the serum calcium[23]. Calcium regulating hormone in kidney decreases the absorption of phosphate and increases its excretion. One of the targets for Vitamin D is bone. When serum calcium is low, it increases osteoclastic activity and stimulates the process of demineralization. In PIH demineralization occurs at a faster rate thus Vitamin D may additionally help in restoring the process of mineralization in pregnant females destined to develop PIH.

In the present study, a significant negative correlation was observed between serum Calcium levels and blood pressure.

Our results are in accordance with the findings of meta-analysis done by Bucher et al in which they found an inverse relationship between blood pressure and calcium supplementation[24]. It has been reported that calcium regulating hormones affect vascular smooth muscle cell. This reduces intracellular ionized calcium and improves the ability of vascular smooth muscle cell to extrude calcium. This in turn decreases the release of rennin and diminishes response to pressor stimuli in pregnant women destined to develop PIH[25]. Direct effect of Vitamin D on rennin angiotensin system has also been

observed. Vitamin D receptor decreases the expression of gene encoding for rennin[26].

Taufield et al have suggested calcium excretion a better parameter to assess the incidence of PIH compared to other serum parameters[27]. In the present study, a significant rise in mean 24 hour urinary calcium and urine calcium /creatinine ratio was seen in Vitamin D supplemented group in comparison to control group. Suarez et al assessed the efficacy of calciuria as a diagnostic test for prediction of preeclampsia. The sensitivity was 80%, specificity 64.8%, positive predictive value 38.7%, negative predictive value 92.1% and the relative risk was 4.9[28].

Urinary calcium represents a balance between glomerular filteration and tubular reabsorption. After vitamin D supplementation in the present study, increased urinary calcium maybe because of raised levels of serum calcium. This increased filtered load of calcium overrides the effect of PTH on tubular reabsorption of calcium. Rodriguez et al, suggested if calcium creatinine ratio <0.04 at 24-34 week in asymptomatic primigravida chances of development of PIH at term increased [29]. In the present study, it has been observed that 18% of subjects in the control group at 36±1week have calcium to creatinine ratio<0.05 and the incidence of PIH was 10%. After vitamin D supplementation, only 6% subjects have calcium to creatinine ratio <0.05 at 36 ±1weeks and incidence of PIH was 4%.

CONCLUSIONS

The investigations support the view that most of the pregnant women prone to develop PIH may have altered calcium homeostasis due to undetected decreased Vitamin D. Administration of Vitamin D in doses of 33000 IU during the third trimester of pregnancy may show efficacious in reducing the incidence of PIH. However, future research is necessary to identify women who stand to benefit most from Vitamin D supplementation during pregnancy. It would be more appropriate to include determination of Vitamin D levels in antenatal protocol before supplementation as it would avoid the side effects related to unnecessary treatment with Vitamin D.

REFERENCES

1. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension. 46: 1243–9, (2005)
2. Merke J, Hofmann W, Goldschmidt D, Ritz E. Demonstration of 1, 25 (OH) 2 vitamin D3 receptors and actions in vascular smooth muscle cells in vitro. Calcif Tissue Int. 41: 112-4, (1987)
3. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA. 294: 2336–41,(2005)
4. Hypponen E. Vitamin D for the prevention of preeclampsia? A hypothesis. Nutr Rev. 63:225-32, (2005)
5. Ingec M, Nazik H, Kadanali S. Urinary calcium excretion in severe preeclampsia and eclampsia. Clin Chem Lab Med. 44(1): 51-3, (2006)
6. McGrowder DA, Williams A, Gordon L, et al. Hypocalciuria in pre-eclampsia and gestational hypertension due to decreased fractional excretion of calcium. Arch Med Sci. 5(1): 80-5, (2009)
7. Ito M, Koyama H, Ohshige A, Maeda T, Yoshimura T, Okamura H. Prevention of preeclampsia with calcium supplementation and vitamin D_3 in an antenatal protocol. Int J Obstet Gynecol. 47: 115-20, (1994)
8. 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. 81:1060–4, (2005)
9. Diaz L, Arranz C, Avila E, Halhali A, Vilchis F, Larrea F. Expression and activity of 25- hydroxyvitamin D-1alpha- hydroxylase are restricted in cultures of human syncytiotrophoblast cells from preeclamptic pregnancies. J Clin Endocrinol Metab. 87: 3876-82, (2002)
10. 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. 92 (9): 3517-22, (2007)

11. World Medical Organization Declaration of Helsinki (1964). BMJ. 313(7070):1448-9, (1996)
12. Marya RK, Rathee S, Lata V, Mudgil S. Effect of Vitamin D supplementation in pregnancy. Gynecol obstet Invest. 12:155-61, (1987)
13. CLSI Document. Procedures for collection of diagnostic blood specimens by venipuncture; Approved standard 27(26)H03-A5, (2006)
14. Hojo M, August P. Calcium metabolism in normal and hypertensive pregnancy. Semin Nephrol. 15(6): 504-11, (1995)
15. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab. 86: 1633–7, (2001)
16. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest.110: 229–38, (2002)
17. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension. 13: 954–9, (1989)
18. Kaur J, Marya RK, Rathee S, Lal H, Singh GP. Effect of pharmacological doses of vitamin D during pregnancy on placental protein status and birth weight. Nutrition Research. 11(9):1077-1081, (1991)
19. Haugen M, Brantsaeter AL, Trogstad L, Alexander J, Roth C, Magnus P, Meltzer HM. Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. Epidemiology. 20(5) : 720-6, (2009)
20. deBoer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). Am J Kid Dis. 50(1): 69-77, (2007)
21. Isakova T, Gutierrez OM, Patel NM, Andress DL, Wolf M, Levin A. Vitamin D deficiency, inflammation and albuminuria in chronic Kidney Disease: Complex Interactions. J Ren Nutr. 21(4): 295-302, (2011)
22. Li YC. Renoprotective effects of Vitamin D analogs. Kidney Int. 78: 134-9, (2010)
23. Bronner F. Intestinal calcium absorption: Mechanisms and applications. J Nutr. 117:1347-52, (1987)
24. Bucher HC, Guyatt GH, Cook RJ, et al. Effect of calcium supplementation on pregnancy-induced hypertension and preeclampsia: a meta-analysis of randomized controlled trials. JAMA. 275: 1113–7, (1996)
25. Hatton DC, McCarron DA. Dietary calcium and blood pressure in experimental models of hypertension. A review. Hypertension. 23 (4):513-30, (1994)
26. Sigmund CD. Regulation of renin expression and blood pressure by vitamin D3. J. Clin. Invest. 110:155–6, (2002)
27. Taufield PA, Ales KL, Resnick LM, Druzin JM, Laragh JH. Hypocalciuria in preeclampsia. N Engl J Med. 316: 715-8, (1987)
28. Suarez VR, Trelles JG, Miyahira JM. Urinary calcium in asymptomatic primigravidas who later developed preeclampsia. Obstet Gynecol. 87: 79-82, (1996)
29. Rodriguez MH, Masaki DI, Mestman J, Kumar D, Rude R. Calcium/ creatinine ratio and microalbuminuria in the prediction of preeclampsia. Am J Obstet Gynecol. 159: 1452-5, (1988)

***Corresponding Author:**
Dr Parul Singla
Deptt of Biochemistry
Sir Ganga Ram Hospital
Old Rajindera Nagar,
New Delhi 110060
Email: *drparul1@yahoo.co.in*