ORIGINAL ARTICLE

Correlation of Vitamin D Levels in Term Normotensive and Pre-eclamptic Patients in Labor

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About the Author

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Abstract

Objective To evaluate maternal vitamin D levels in term normotensive and preeclamptic patients in labor and to assess additional factors such as maternal and cord blood levels of calcium, phosphorus, parathormone, and alkaline phosphatase and associated factors such as BMI, birth weight, and mode of delivery.

Method This was a case control study carried out in Department of Obstetrics and Gynaecology, ESIC-PGIMSR, New Delhi, India from August 2012–April 2014. A total of 100 patients were divided into two equal groups (control and study groups of 50 each). Control group had women with singleton uncomplicated, term normotensive pregnant women in labor while the study group composed of term preeclamptic women in labor. Blood samples were drawn for vitamin D, serum calcium, serum phosphorus, serum alkaline phosphatase, and serum parathormone levels during first stage of labor, and subsequently, their levels were evaluated in cord blood also.

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Results All the enrolled patients had vitamin D deficiency pointing toward a universal prevalence of this micronutrient deficiency in antenatal patients. We found more incidence of severe vitamin D deficiency (90 %) in preeclamptic patients as compared to normotensive patients (62 %). Also preeclamptic group had lower median vitamin D levels (3.9 ng/ml) when compared to normotensive group (9 ng/ml). Similarly, all the neonates were found to be vitamin D deficient as assessed by their cord blood levels. Neonates born to preeclamptic mothers had lower median cord blood vitamin D levels (4.4 ng/ml) when compared to those born to normotensive mothers (7.25 ng/ml). The mean maternal calcium levels followed trends observed in vitamin D levels with preeclamptic patients having consistently lower calcium levels (mean value of 8.50 ± 0.80 mg/dl) when compared to normotensive patients (mean value of 8.89 ± 0.56 mg/dl). Preeclamptic group was found to have more number of patients (58 %) with higher BMI when compared to normotensive group (32 % of patients). A slightly more incidence (36 %) of low birth weight babies is being born to preeclamptic mothers as compared to normotensive mothers (34 %). Significantly a more number of patients (36 %) with vitamin D levels below 15 ng/ml underwent cesarean section when compared to only 9 % of patients having vitamin D level above this level.

Conclusion Preeclampsia is indeed associated with lower vitamin D levels, and its pathophysiology involves vitamin D and calcium metabolism.

Keywords Vitamin D · Preeclampsia · Vitamin D deficiency

Introduction

Vitamin D deficiency during pregnancy has been linked with a number of adverse outcomes. Preeclampsia is a pregnancy-specific syndrome that affects approximately 3–7 % of first pregnancies. The known racial disparity in preeclampsia, with black women being more likely to develop severe preeclampsia and suffer greater morbidity associated with the disorder than white women, suggests that vitamin D may be relevant.

In the general population, 25-hydroxyvitamin D (25(OH)D) deficiency has been linked to hypertension [1–3]. In preeclampsia, the most severe form of gestational hypertension, studies have consistently found alterations in calcium and vitamin D metabolism during clinical disease in late pregnancy; these include hypocalciuria and low serum 1,25-dihydroxyvitamin D $(1,25(OH)_2D)$.

One of the first study to explore the 25(OH)D-preeclampsia relation before the onset of clinical symptoms was conducted by Bodnar et al. [4] in the year 2007. They conducted a nested case–control study and reported lower early pregnancy levels of 25(OH)D in women who later developed preeclampsia, suggesting that 25(OH)D deficiency antedates disease onset and might contribute to its pathogenesis.

Early pregnancy maternal 25(OH)D less than 37.5 nmol/liter was associated with a fivefold increase in the odds of preeclampsia, independent of race/ethnicity, season, sample gestational age, pre pregnancy BMI, and education. At delivery, maternal 25(OH)D concentrations remained 15 % lower in women with overt preeclampsia compared with non-preeclamptic controls. Their results showed that maternal vitamin D deficiency at less than 22 weeks gestation was a strong, independent risk factor for preeclampsia. Importantly, there was a monotonic dose–response relation between maternal serum 25(OH)D and risk of preeclampsia. There was a high correlation between maternal predelivery and cord serum 25(OH)D in cases, and therefore, cord serum 25(OH)D concentrations were also significantly lower among neonates of preeclamptic mothers than among neonates of non-preeclamptic control mothers. These differences were found in their population despite widespread prenatal/multivitamin use in the 3 months before delivery (93 %) and in the periconceptional period (46 %).

Methodology

This study was conducted in the Department of Obstetrics and Gynaecology, ESIC-PGIMSR, Basaidarapur, New Delhi. In our study, 100 patients who gave informed written consent were enrolled. The patients were divided equally into study and control groups of 50 each. In the study group, patients having preeclampsia (defined as BP $\geq$140/90 mm Hg after 20 weeks of gestation and proteinuria $\geq$1 + dipstick) at term in labor were enrolled whereas normotensive patients (defined as BP <140/90 after 20 weeks of gestation and proteinuria <1 + dipstick) at term in labor were included in the control group. On admission, brief history and clinical examination were done. In all the patients, blood samples for vitamin D, serum calcium, serum phosphorus, serum parathormone, and serum alkaline phosphatase levels were drawn during first stage of labor, and subsequently, their levels were evaluated in cord blood also.

Vitamin D deficiency was defined as 25(OH)D levels below 15 ng/ml [5]. Severe vitamin D deficiency was defined as 25(OH)D levels below 10 ng/ml [6]. Additional factors such as BMI, age, mode of delivery, and birth weight of baby were also studied in both the groups.

Results

All the patients enrolled in both the study and control group were found to be vitamin D deficient. The maternal vitamin

D levels in the study group were relatively lower with a median value of 3.9 ng/ml as compared to 9.0 ng/ml in the control group. This difference in the median maternal vitamin D levels of both the groups was found to be statistically significant with p value of <0.001. 90 % of the patients in the study group were found to be severely vitamin D deficient (vitamin D levels <10 ng/ml) as compared to 62 % of the patients in the control group. This difference in the number of patients with severe vitamin D deficiency among the two groups was also statistically significant with p value of 0.005 (Table 1; Fig. 1).

Neonates in both the study and the control group were vitamin D deficient. The median cord blood vitamin D levels in the study group were 4.40 ng/ml while it was 7.25 ng/ml in the control group. The difference in the median cord blood vitamin D levels of both the groups was found to be statistically significant with p value of 0.001. 90 % of the neonates in the study group were found to be severely vitamin D deficient (vitamin D levels <10 ng/ml) as compared to 66 % of the neonates in the control group. This difference in the number of neonates with severe vitamin D deficiency among the two groups was also statistically significant with p value of 0.006. A higher number of neonates born to preeclamptic mothers were severely vitamin D deficient when compared to those born to normotensive mothers (Table 2; Fig. 2).

The mean maternal calcium levels were relatively lower in the study group (8.50 ± 0.80) mg/dl as compared to those in the control group (8.89 ± 0.55) mg/dl. The difference in the mean maternal calcium levels of both the groups was found to be statistically significant with p value of 0.006. A more number of patients (44 %) in the study group were having low calcium levels (<8.5 mg/dl) as compared to the control group (22 %). This difference in the two groups in terms of number of patients with low calcium levels was found to be statistically significant with p value of 0.019 (Table 3; Fig. 3).

Cord blood calcium levels closely followed the results obtained for maternal calcium levels with neonates born to preeclamptic mothers having lower mean calcium value (8.92 ± 1.03 mg/dl) as compared to values (9.64 ± 0.84 mg/dl) found in neonates born to normotensive mothers.
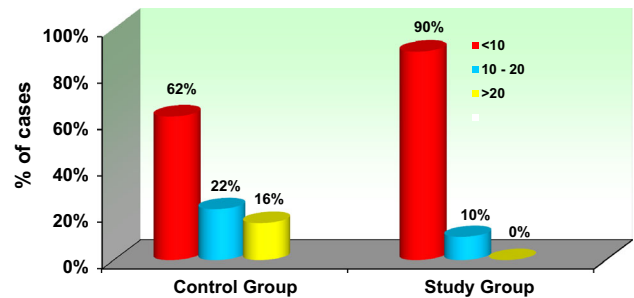

Fig. 1 Shows comparison of maternal vitamin D levels (ng/ml) between study and control group

Maternal and cord blood phosphorus levels, and alkaline phosphatase and parathormone levels showed slight variations when compared in preeclamptic and normotensive mothers. These variations were not found to be statistically significant so as to draw a conclusion.

34.8 % of neonates born to vitamin D deficient (<15 ng/ml) mothers were low birthweight when compared to 36.3 % of neonates born to mothers having vitamin D levels more than or equal to 15 ng/ml. The difference in the percentage of low birth weight neonates born to vitamin D deficient mothers and mothers with vitamin D levels ≥15 ng/ml was not significant (p value of 1.0) (Table 4; Fig. 4).

Discussion

Bodnar et al. [4] suggested that vitamin D deficiency in early pregnancy is an independent risk factor for preeclampsia and recommended vitamin D supplementation in early pregnancy as a safe and effective means of preventing preeclampsia. The biochemical basis of the role of vitamin D in pathophysiology of preeclampsia has been explained by Evans et al. [7], Daftary et al. [8], and Braam et al. [9] who found vitamin D_3 to regulate the transcription and function of genes associated with placental invasion, normal implantation, and angiogenesis. Furthermore, defective implantation of placenta, which is central to the

**Table 1** Showing comparison of maternal vitamin D levels (ng/ml) between study and control group

Vitamin D levels (ng/ml)	Control group		Study group		p value
	No. of cases	%	No. of cases	%	
<10	31	62	45	90	0.005*
10–20	11	22	5	10	
>20	8	16	0	0	
Total	50	100	50	100	
Median (IQR)	9.0 (6.45–12.75)		3.90 (3.07–6.0)		<0.001*

* Statistically significant

Table 2 Showing comparison of cord blood vitamin D levels between study and control group

Cord blood vitamin D levels (ng/ml)	Control group		Study group		p value
	No. of neonates	%	No. of neonates	%	
<10	33	66	45	90	0.006*
10–20	11	22	5	10	
>20	6	12	0	0	
Total	50	100	50	100	
Median (IQR)	7.25 (4.0–13.0)		4.40 (3.20–7.40)		0.001*

* Statistically significant
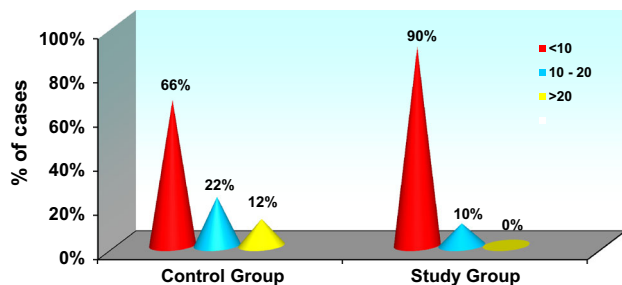
Fig. 2 Shows comparison of cord blood vitamin D levels (ng/ml) between study and control group
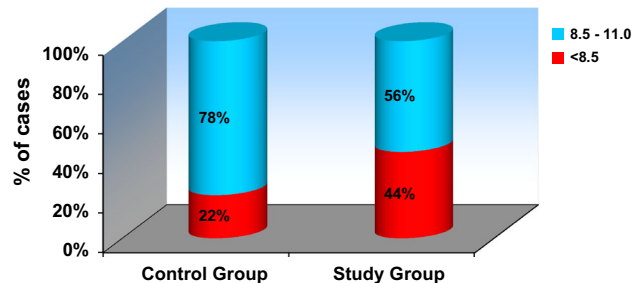
Fig. 3 Shows comparison of maternal calcium levels (mg/dl) between study and control group

development of preeclampsia, has been found to be mediated by an inappropriate immune response between mother and baby.

Another mechanism for development of preeclampsia has been reported by Bodnar et al. [4] who stated that development of preeclampsia may be mediated by renal vascular endothelial growth factor (VEGF) which promotes proteinuria. Further, vitamin D_3 was found to have a regulating effect on angiogenic processes through direct influence on VEGF gene transcription. Thus, their study showed that vitamin D could be a risk factor for the development of preeclampsia.

In contrast to above findings in a nested case control study, Powe et al. [10] found no association between first trimester total 25(OH)D and subsequent preeclampsia. They stated that first trimester 25(OH)D deficiency is not a prevalent cause of preeclampsia. They further reported that

vitamin D binding protein (VDBP) and calculated free levels of 25(OH)D were also not altered in the first trimester among women who later developed preeclampsia. They made a significant finding that conditions classically associated with lower levels of total 25(OH)D, including obesity, were not associated with alterations in calculated free 25(OH)D levels.

But importantly even Powe et al. [10] agreed that at very low levels of 25 hydroxyvitamin D, there was a significant association between 25 hydroxyvitamin D and occurrence of preeclampsia.

Neonates in both the study and the control group were vitamin D deficient. The results obtained for cord blood vitamin D levels closely followed those for the maternal vitamin D. Very similar results were reported by Bodnar et al. [4] who found that cord serum 25(OH)D concentrations were significantly lower among neonates of

Table 3 Showing comparison of maternal calcium levels between study and control group

Calcium levels (mg/dl)	Control group		Study group		p value
	No. of cases	%	No. of cases	%	
<8.5	11	22	22	44	0.019*
8.5–11.0	39	78	28	56	
>11.0	0	0	0	0	
Total	50	100	50	100	
Mean ± SD	8.89 ± 0.55		8.50 ± 0.80		0.006*

* Statistically significant

Table 4 Showing relation between maternal vitamin D levels and birth weight

Birth weight (kg)	Vitamin D levels <15 ng/ml		Vitamin D levels ≥15 ng/ml		p value
	No. of cases	%	No. of cases	%	
<2.5	31	34.8	04	36.3	1.000
≥2.5	58	65.2	07	63.6	
Total	89	100	11	100	
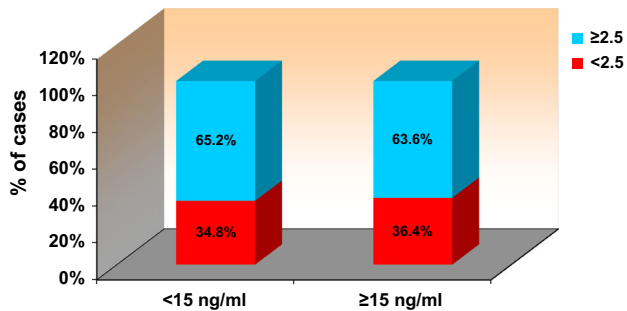
Fig. 4 Showing relation between maternal vitamin D levels and birth weight

preeclamptic mothers than among neonates of normotensive control mothers.

The mean maternal calcium levels were relatively lower in the study group (8.50 ± 0.80) mg/dl as compared to those in the control group (8.89 ± 0.55) mg/dl. The difference in the mean maternal calcium levels of both the groups was found to be statistically significant with p value of 0.006.

Results from various studies by Kumru et al. [11], Adam et al. [12], Joshi et al. [13], and Sukonpan et al. [14] have shown the variation in serum levels of calcium, magnesium, and zinc in pregnant patients with respect to their blood pressure values. They found a consistent fall in their levels in hypertensive pregnant women. This decline is in addition to the fact that during pregnancy, there is a progressive decline in concentration of calcium, magnesium, and zinc in maternal serum possibly due to hemodilution, increased urinary excretion, and increased transfer of these minerals from the mother to the growing fetus. Serum levels of calcium, in turn, seems to play a part in the development of preeclampsia, as it has been seen that a reduction in serum calcium level causes increase in release of parathyroid hormone and renin, which in turn causes increase in intracellular calcium in vascular smooth muscle. This increase in intracellular calcium in vascular smooth muscle causes increased vascular resistance and vasoconstriction, thus leading to rise in blood pressure. In addition, low dietary intake and accelerated metabolism might be the other contributing factors.

Belizan and Villar [15] have shown the inverse relationship between calcium intake and hypertension during pregnancy. Similar findings were further supported by other studies by Hamlin [16], Villar et al. [17], Belizan et al. [18], and Jain et al. [19].

Infants born at term to mothers with preeclampsia have similar birth weights, on average, to those of infants born to normotensive mothers. These findings are in concurrence with the observation made by Xiong et al. [20] in their study in 2002 who stated that preeclampsia did not necessarily amount to low birth weight.

34.8 % of neonates born to vitamin D deficient (<15 ng/ml) mothers were low birth weight when compared to 36.3 % of neonates born to mothers having vitamin D levels more than or equal to 15 ng/ml. Though this difference in the percentage of low birth weight neonates born to vitamin D deficient mothers and mothers with vitamin D levels ≥15 ng/ml was not significant, but still in line with what has been mentioned in earlier literature, that there has always been noted a higher incidence of low birth weight neonates in vitamin D deficient mothers. Bodnar and Simhan [21] found that maternal vitamin D deficiency may be a risk factor for severe preeclampsia but not for its mild subtypes. Wei et al. [22] and Baker et al. [23] found that maternal midgestation vitamin D deficiency was associated with increased risk of severe preeclampsia.

Ullah et al. [24] evaluated potential risk of vitamin D deficiency for both preeclampsia and eclampsia and found that the odds of developing preeclampsia and eclampsia may increase by up to 5-fold in women with vitamin D insufficiency. Since preeclampsia and eclampsia can lead to serious complications for both mother and the offspring, vitamin D may be supplemented during pregnancy in high risk populations to decrease these adverse consequences.

At present, it is not known whether maternal vitamin D levels track over gestation, since results obtained from the investigation of vitamin D in gestation are inconclusive. It is not known that which stage in pregnancy is most relevant, but it is possible that an association between 25(OH)D concentration and birth weight is prevalent when 25(OH)D concentration is measured early in pregnancy, the stage at which growth trajectory is set and bone development starts.

Vitamin D plays an important role in a range of health-related processes, and the poor vitamin D status in several ethnic minority groups indicates the beneficial role of extra vitamin D on an individual level, not only to improve a woman's health but possibly also to improve that of her child, both before and after birth.

Conclusion

Preeclampsia is indeed associated with lower vitamin D levels, and its pathophysiology involves vitamin D and calcium metabolism. Vitamin D deficiency may be a modifiable risk factor for severe preeclampsia. The present study can serve as a starting point for larger randomized controlled studies with more number of patients so as to validate the results obtained so far.

Compliance with ethical requirements and Conflict of interest   All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study. An ethical clearance has also been taken from the institutional ethical committee. The authors of the article Gupta T, Wahi S, Gupta N, Arora S, Gupta S, Bhatia P declare that they have no conflict of interest.

References

1. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin d levels and risk of incident hypertension. Hypertension. 2007;49:1063–9.
2. Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin d in the united states: Data from the third national health and nutrition examination survey. Arch Intern Med. 2007;167:1159–65.
3. Melamed ML, Michos ED, Post W, et al. 25-hydroxyvitamin d levels and the risk of mortality in the general population. Arch Intern Med. 2008;168:1629–37.
4. Bodnar LM, Catov JM, Simhan HN, et al. Maternal vitamin D deficiency increases the risk of pre eclampsia. Clin J Endocrinol Metab. 2007;92:3517–22.
5. Merewood A, Mehta SD, Chen TC, et al. Association between vitamin D deficiency and primary cesarean section. J ClinEndocrinolMetab. 2009;94(3):940–5.
6. Sahu M, Das V, Agarwal A, et al. Vitamin D replacement in pregnant women in rural north india: a pilot study. Eur J Clin Nutr. 2009;63:1157–9.
7. Evans KN, Bulmer JN, Kilby MD, et al. Vitamin D and placental-decidual function. J SocGynecolInvestig. 2004;11:263–71.
8. Daftary GS, Taylor HS. Endocrine regulation of HOX genes. Endocr Rev. 2006;27:331–55.
9. Braam LA, Hoeks AP, Brouns F, et al. Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. ThrombHaemost. 2004;91:373–80.
10. Powe CE, Seely EW, Rana S, et al. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension. 2010;56(4):758–63.
11. Kumru S, Aydin S, Simsek M, et al. Comparison of serum copper, zinc, calcium, and magnesium levels in pre-eclamptic and healthy pregnant women. Biol Trace Elem Res. 2003;94:105–12.
12. Adam B, Malatyalioglu E, Alvur M, et al. Magnesium, zinc and iron levels in pre-eclampsia. J Matern Fetal Med. 2001;10:246–50.
13. Joshi VK, Sapre S, Govilla V. Role of micronutrients and calcium in pregnancy induced hypertension. Obs Gynae Today. 2003;8:617–9.
14. Sukonpan K, Phupong V. Serum calcium and serum magnesium in normal and pre-eclamptic pregnancy. Arch Gynecol Obstet. 2005;273:12–6.
15. Belizan JM, Villar J. The relationship between calcium intake and edema, proteinuria and hypertension-gestosis: a hypothesis. Am J Clin Nutr. 1980;33:2202–10.
16. Hamlin RH. The prevention of eclampsia and pre-eclampsia. Lancet. 1952;1:64–8.
17. Villar J, Belizan JM, Fischer PJ. Epidemiologic observations on the relationship between calcium intake and eclampsia. Int J Gynaecol Obstet. 1983;21:271–8.
18. Belizan JM, Villar J, Repke J. The relationship between calcium intake and pregnancy-induced hypertension: up-to-date evidence. Am J Obstet Gynecol. 1988;158:898–902.
19. Jain S, Sharma P, Kulshreshtha S, et al. The role of calcium, magnesium, and zinc in pre-eclampsia. Biol Trace Elem Res. 2010;133:162–70.
20. Xiong X, Demianczuk NN, Saunders LD, et al. Impact of pre-eclampsia and gestational hypertension on birth weight by gestational age. Am J Epidemiol. 2002;155(3):203–9.
21. Bodnar LM, Simhan HN, Catov JM. Maternal vitamin D status and the risk of mild and severe preeclampsia. Epidemiology. 2014;25(2):207–14.
22. Wei SQ, Audibert F, Hidiroglou N, et al. Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. BJOG. 2012;119(7):832–9.
23. Baker AM, Haeri S, Camargo CA Jr, et al. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. J Clin Endocrinol Metab. 2010;95(11):5105–9.
24. Ullah MI, Koch CA, Tamanna S, et al. Vitamin D deficiency and the risk of preeclampsia and eclampsia in Bangladesh. Horm Metab Res. 2013;45(9):682–7.