ORIGINAL RESEARCH

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Vitamin D and hypertension in pregnancy

Abstract

Purpose: Vitamin D Deficiency is common, particularly in northern latitudes. We examined the association between vitamin D status and hypertension in late pregnancy.

Methods: A case-control study was conducted during two time periods: September-October, 2008, and January-March, 2009, in women near term. A case was defined as having two or more documented blood pressure readings above 140/90 (either/or) at any time during pregnancy (n=78). Controls had at least two blood pressure readings, with none above 140/90 during pregnancy (n=109). Serum 25-hydroxyvitamin D (25(OH)D) was measured in all participants.

Results: In the summer, 13% of controls and 29% of the cases had 25(OH) D levels < 50 nmol/L. During the winter, these numbers rose to 44% and 49% respectively. Both cases and controls were more likely to be vitamin D deficient in the winter (p=0.002). There was a negative correlation between BMI and 25(OH)D (r=-0.202, p=0.002). In univariate analysis, cases had lower 25(OH)D (p=0.046), but also higher body mass index, so that in multivariate analysis 25(OH)D status was no longer significant. There was no difference in mean oral daily vitamin D intake (dietary intake and supplements, 746 and 785 IU respectively). Controls gained less weight in pregnancy. There was a negative correlation between the highest blood pressure measured in pregnancy and 25(OH)D levels (r= -0.118; p=0.012).

Conclusion: There is a high prevalence of vitamin D deficiency in pregnant women recruited in Saskatoon, Saskatchewan. Women with low circulating vitamin D concentrations are more likely to have hypertension.

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Vitamin D deficiency is common, particularly in northern latitudes where winter sunlight is insufficient to allow vitamin D synthesis [1,2,3]. Saskatoon, Saskatchewan is located at 52 degrees North, the period of ineffective vitamin D synthesis extends from October through March [3]. Adequate 25-hydroxyvitamin D 25(OH)D concentrations are necessary for maximal calcium absorption; with lower levels, parathyroid hormone concentration increases. The definitions of vitamin D deficiency vary, but center around the plasma concentration of 25(OH)D. One accepted definition, based on the 25(OH) D level below which parathyroid hormone level increases, defines deficiency as less than 50 nmol/L, and insufficiency as 52-72 nmol/L [4,5]. Using these definitions, Holick estimates that 1 billion people worldwide are vitamin D deficient [5]. One New Zealand (latitude 35-47 degrees South) study reported that 87% of pregnant women were vitamin D deficient [6].

The consequences of vitamin D deficiency and insufficiency are numerous. In recent literature, low levels of vitamin D have been linked to cardiovascular disease [7-9], hypertension [10-12], wheezing illness in children [13], cancer, multiple sclerosis, type 1 diabetes, Crohn's disease and osteoporosis [5]. In pregnancy, low levels of vitamin D have been associated with pre-eclampsia [14,15], insulin resistance [16], low birth weight [17,18], and primary cesarean section [19].

There is epidemiological evidence to support the association between hypertension and vitamin D deficiency. There is a higher prevalence of hypertension in northern latitudes [5,20]. Blood pressure has been demonstrated to be higher in winter compared with summer [20]. Vitamin D supplementation decreased blood pressure in some studies [10] but not others [21]. Plasma 25(OH)D levels have been shown to be inversely associated with the risk of incident hypertension [11,22]. The mechanisms proposed include increased parathyroid hormone causing resistance vessel hypertrophy [23], reduced suppression of kidney renin gene expression by 1,25(OH)D and direct effects of vitamin D on the vasculature [7].

Given the high prevalence of vitamin D deficiency in pregnancy and the potential consequences of this deficiency, we set out to determine the prevalence of vitamin D deficiency in women presenting to Royal University Hospital in Saskatoon, Saskatchewan for delivery and whether there was a difference in this prevalence during the winter compared with summer months. We hypothesized that vitamin D levels would be lower in women with hypertension compared with their non-hypertensive peers. We further hypothesized that this difference would be exaggerated in the winter compared with summer months.

Methods

A case-control study was conducted during two time periods: September 2-October 3, 2008 (summer); and January 19-March 6, 2009 (winter). All deliveries in the Saskatoon Health Region and those referred from other regions are performed at Royal University Hospital. The protocol was designed and written by the authors and approved by the Institutional Review Board at the University of Saskatchewan and by the Saskatoon Health Region. The data were analyzed by the authors and the Clinical Research Support Unit at the University of Saskatchewan. The authors vouch for the accuracy and completeness of the data and analyses.

Study Population

All women presenting to the hospital prior to, or immediately after delivery were eligible to participate. Women with type 1 diabetes, chronic kidney disease, parathyroid disease, delivery at < 24 weeks gestational age, those carrying fetuses with life-threatening congenital anomalies, and those unable or unwilling to give consent were excluded from the study.

A case was defined as having two or more documented blood pressure readings above 140/90 (either/or) at any time during pregnancy. Blood pressure readings from either the prenatal record or the hospital chart were used. Controls were women who had at least two readings, with none above this threshold. Blood pressure readings during labour were not accepted. Women who had only one reading above 140/90 or less than two blood pressure readings in total were not further analyzed.

Study Protocol

Informed consent was obtained from participants. Demographic information and historical medical and obstetrical information were obtained. Standard peri-partum blood test results were recorded. Ante-partum and post-partum blood pressures were taken with automated equipment. Early pregnancy and admission maternal weights were recorded. Infant weight, gender, cord blood pH and Apgar scores were documented.

Blood samples for calcium, albumin and 25 (OH) D were obtained within 72 hours of delivery and were analyzed at the Hospitals-in-Common Laboratory in Toronto. The vitamin D assay used was Liaison®, while the calcium and albumin assays were Roche Modular. Women for whom blood samples were not obtained were excluded from the analysis (see Figure 1).

A food frequency questionnaire was also administered. This questionnaire has been previously validated against both 7-day food diary and 25(OH) D levels [24].

FIGURE 1. Study recruitment and enrollment.

Statistical Analysis

In calculating sample size requirements, we estimated that 30% of controls and 55% of cases would have 25(OH) D blood concentrations under 50 nmol/L. This conservative assumption was based on estimates from previous studies [6,14]. Therefore 68 patients per group were required to achieve a power of 80% and an α of 0.05.

In a secondary analysis we sought a correlation between 25(OH)D concentration and the highest systolic blood pressure recorded, regardless of the assignment to Case or Control group.

A number of potential confounders thought to be associated with pregnancy induced hypertension including: body mass index (kg/m^2), early pregnancy blood pressure, parity, type 2 or gestational diabetes, weight gain in pregnancy and gestational age at delivery.

Finally, we reasoned that plasma 25(OH)D concentrations would be lower in the winter months than in September, so performed a pre-specified analysis by season.

Study subject characteristics were described using frequencies for categorical variables and means with standard deviation for continuous variables. Univariate analysis of categorical variables were analyzed using chi-square testing. Fisher's exact test was utilized if fewer than five outcomes were available. Paired t-testing was utilized for continuous variables. The multivariate analysis was completed using logistic regression for all variables with $p < 0.1$ in the univariate analysis with hypertension as the dependent variable. Correlation between BMI and 25 (OH) D levels was tested using the Pearson coefficient. All of the analysis were 2-sided and the level of statistical significance was defined as $\alpha = 0.05$. Statistical analysis was completed using SPSS version 17.0 and SAS version 9.2.

Results

There were 1041 deliveries at Royal University Hospital during our two recruitment periods (September 2-October 3, 2008 and January 19-March 6, 2009). Due to short length of stay of many parturients, we were unable to contact all, but 302 potential participants (139 in summer and 163 in winter) were approached for involvement in the study. Informed consent was obtained from 242 patients (123 in summer and 119 in winter). Of these, 55 patients excluded from the analysis for various reasons (patients with only diabetes and not hypertension, patients whose blood was not drawn, patients who had chronic kidney disease, or patients who had only one blood pressure reading $>140/90$). Thus, 78 cases and 109 controls were included in our analysis (Figure 1).

TABLE 1. Univariate analysis

Variable	Cases*	Controls*	p	95% c.i.†
n	78	109		
25(OH)D < 50 nmol/L, # (%)	30 (38)	32 (29)	0.210	0.9-3.1
Winter # (%)	41 (53)	55 (50)	0.899	0.49-1.56
Age (yr)	29.1 $\pm$ 5.8	29.0 $\pm$ 5.0	0.875	0.95-1.06
Primipara # (%)	65 (83)	34 (32)	0.031	0.29-0.94
BMI 1 st visit (Kg/m ²)	31.1 $\pm$ 7.5	25.1 $\pm$ 7.2	0.017	1.07-1.91
Weight gain (Kg)	18.0 $\pm$ 9.5	15.7 $\pm$ 7.1	0.001	1.0-1.08
SBP 1 st visit‡	125 $\pm$ 15.4	111.4 $\pm$ 10.1	0.001	1.06-1.13
DBP 1 st visit§	74.4 $\pm$ 8.9	66.0 $\pm$ 7.4	0.001	1.09-1.19
GDM (yes, %)	12 (23.5)	0 (0)	0.001	
Type 2 DM? (yes,%)	2 (3.9)	0 (0)	0.173	
Smoke? (yes,%)	12 (15.4)	17 (15.6)	1.000	0.45-2.04
Ca ⁺⁺ (mmol)	2.1 $\pm$ 0.2	2.2 $\pm$ 0.2	0.893	0.6-24.6
Albumin (g/L)	29.2 $\pm$ 3.7	30.5 $\pm$ 3.4	0.755	0.83-0.98
25 (OH)D (nmol/L)	61.7 $\pm$ 25.9	69.9 $\pm$ 28.5	0.046	0.98-1.00
Total Vit D intake (IU)	785 $\pm$ 362	746 $\pm$ 344	0.550	0.99-1.00
Ca ⁺⁺ intake (mg/d)	1765 $\pm$ 822	1672 $\pm$ 665	0.625	0.99-1.00
Gestational age at delivery (weeks)	38.4 $\pm$ 2.3	39.4 $\pm$ 1.9	0.002	0.65-0.91
Infant weight	3271 $\pm$ 767	3375 $\pm$ 501	0.250	
Infant gender			0.76	
Male	33	54		
Female	45	55		
Apgar 1 minute	7.64 $\pm$ 1.58	7.79 $\pm$ 1.58	0.52	
Apgar 5 minute	8.79 $\pm$ 0.77	8.82 $\pm$ 0.58	0.81	
Venous cord pH	7.32 $\pm$ 0.05	7.34 $\pm$ 0.06	0.001	
Arterial cord pH	7.26 $\pm$ 0.05	7.27 $\pm$ 0.06	0.28	

* $\pm$ standard deviation

† 95 % c.i.: 95% confidence interval

‡ SBP 1st Visit-systolic blood pressure at first pre-natal visit

§ DBP 1st Visit-diastolic blood pressure at first pre-natal visit

|| GDM-gestational diabetes

FIGURE 2. 25-hydroxyvitamin D (25(OH)D) concentration by season.

Table 1 outlines the main results of the study from the univariate analysis; including demographic maternal characteristics, fetal characteristics, maternal blood pressures, serum calcium and 25(OH) D levels in addition to calcium and vitamin D intake. Overall, the serum vitamin D levels were low. In the summer, 13% of the control group and 29% of the cases had 25(OH) D levels < 50 nmol/L, the level that defines vitamin D deficiency. During the winter testing time, these numbers rose to 44% and 49% respectively. Both cases and controls were more likely to be vitamin D deficient in the winter ($p=0.002$). Serum 25(OH)D concentrations in the case group were lower compared with the control group in the univariate analysis ($61.7 \text{ nmol/L} \pm 25.9$; $69.9 \text{ nmol/L} \pm 28.5$ respectively $p=0.046$). This difference was more marked in the summer (Figure 2).

The control group had lower BMI than the cases and less weight gain in pregnancy ($p=0.017$ and $p=0.001$ respectively). There was a negative correlation between BMI and 25(OH)D

TABLE 2. Multivariate analysis

Variable	Odds Ratio*	p	95% c.i.†
Parity (≥ 1)	0.523	0.031	0.29-0.94
BMI >30	3.57	0.0001	1.87-6.8
Body Surface Area	26.1	<0.0001	5.78-117.9
Weight Gain	1.04	0.067	1.00-1.08
SBP 1 st ‡	1.09	<0.0001	1.06-1.13
DBP 1 st §	1.14	<0.0001	1.09-1.19

* Odds ratio: cases vs controls

† 95% confidence interval

‡ SBP 1st Visit-systolic blood pressure at first pre-natal visit

§ DBP 1st Visit-diastolic blood pressure at first pre-natal visit

levels ($r=-0.202$, $p=0.002$); however, in the multivariate analysis, which corrected for BMI, the difference in 25(OH)D was no longer significant (Table 2). In the univariate analysis, there was a modest negative correlation between the highest blood pressure measured in pregnancy and 25(OH)D levels ($r=-0.118$; $p=0.012$). Again, this was not seen in the multivariate analysis. The traditional predictors of gestational hypertension: BMI, parity, initial blood pressure were confirmed. (Table 2) Vitamin D intake from food and supplements was not different between groups (Table 1).

The women in the case group delivered at a slightly earlier gestational age ($p=0.002$) than the control group.

Discussion

These data reveal a significantly lower 25(OH)D level in the case group compared with the control group on univariate analysis. This effect was not observed on the multivariate analysis. There are several possible explanations for this. First, the overall vitamin D levels were very low and the range relatively small, which may have made differences between the groups difficult to detect. The summer data, where the average vitamin D level in the control group was in the vitamin D sufficiency range ($>75 \text{ nmol/L}$), did show significantly lower vitamin D levels in the case group compared with the control group. This suggests that vitamin D sufficiency may protect against gestational hypertension. Secondly, the multivariate analysis was dominated by the strength of the BMI effect on predicting gestational hypertension. People with elevated BMI have lower 25(OH)D levels [25]. One possible reason is simply the depot effect of a large adipose mass for this fat soluble vitamin, but other possibilities exist including lower outdoor physical activity. This negative correlation was observed in our data and dominated the multivariate analysis. Thirdly, only about a third of the total number of patients delivered during the time periods were enrolled in the study. This was primarily due to the

early discharge policy of our Region. Likely, those not studied were a healthier group, as hypertension is a major reason to delay discharge. It is possible that these healthy patients had higher 25(OH)D levels that protected them from hypertension.

Cases and controls were defined solely on the basis of recorded blood pressure readings. About one third of the patients we approached did not fall into either category. This reduced our sample size and our ability to discern true differences. Had we defined cases more stringently, as hypertension occurring after 20 weeks gestation with or without proteinuria, we would have reduced our sample size even further. Ideally, women with essential hypertension, gestational hypertension and preeclampsia would all be included in our case group. Although these are all distinct entities, the number of women with essential hypertension in this age group would be small [26] and that the women with gestational hypertension and those with preeclampsia may have the same pathology of varying degree.

The mechanism by which vitamin D might protect against hypertension in pregnancy is uncertain. Indeed, the pathogenesis of gestational hypertension and pre-eclampsia is unknown. One theory holds that poor placentation leads to placental ischemia, which, in turn, causes the release of cytokines including soluble fms-like tyrosine kinase 1 (sFlt-1) [27]. This binds to, and inactivates, vascular endothelial growth factor (VEGF). VEGF normally increases endothelial prostacyclin and nitric oxide release; when VEGF levels are deficient, their normal vasodilatory and antithrombotic effects are lost. Hypertension and renal thrombotic microangiopathy result [28]. The immunomodulatory effects of vitamin D could make successful placentation more likely [29,30]. In all pregnancies, maternal-conceptus cross talk is important in the preparation of the uterus and the development of the blastocyst [30]. Vitamin D may play an important role in this cross talk [30]. Both deciduas and trophoblast cells express vitamin D receptors in addition to 1α -OHase, which suggests an autocrine or paracrine role for $1,25(\text{OH})_2\text{D}$ within these tissues [30,31]. This role may be immunosuppressive or may influence implantation [30].

In this regard, it is interesting that a seasonal difference in pre-eclampsia has been observed with the higher risk of preeclampsia occurring in winter months [32]. There is also some evidence that gestational blood pressure is higher in winter and lower in summer [33].

The larger difference that we observed between 25(OH) D levels in the cases compared with controls in the summer testing time (Figure 2) may reflect a difference in vitamin D expo-

sure in early pregnancy, maintained to the end of pregnancy or a difference acquired near the time of delivery. Powe [34] and Shand [35] and their colleagues found no predictive value of low first trimester 25(OH)D levels for gestational hypertension. Thus, there remains a lack of evidence as to correct dosage of vitamin D as well as the timing of starting supplements. A randomized controlled trial of vitamin D supplementation would seem reasonable.

The high prevalence of 25(OH) D deficiency in pregnancy occurs despite the widespread use of pre-natal vitamins [36]. Prenatal vitamins typically contain 400 IU of Vitamin D. The current Canadian Pediatric Society recommendations indicate that "consideration should be given to administering 2000 IU of vitamin D daily to pregnant and lactating women, especially during the winter months, to maintain vitamin D sufficiency. The effectiveness of this regimen and possible side effects should be checked with periodic assays for 25(OH) D and calcium (recommendation grade A)." [37] The current Health Canada recommendations are for 600 IU of vitamin D daily during pregnancy and lactation [38].

Conclusion

Our study reinforces the high prevalence of vitamin D deficiency in pregnant women. Recent research has demonstrated the importance of vitamin D sufficiency during pregnancy and beyond. In Saskatoon, sunlight-induced vitamin D synthesis is ineffective between October and March. Even during a period where effective vitamin D synthesis was possible, 13-29% of our study participants were vitamin D deficient. Mean vitamin D intake (including dietary intake and supplements) was 746-785 IU. Our data suggest that the Health Canada recommended daily intake (600 IU) [38] may not be enough to avoid vitamin D deficiency in pregnancy and lactation. Future research is needed to determine the effect of vitamin D supplementation on the incidence of gestational hypertension and preeclampsia.

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