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Even in Vitamin D Deficiency 1, 25 Dihydroxyvitamin D3 Level Is Normal in Pregnant Women

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Abstract

Objectives: Vitamin D deficiency is a controversial issue in pregnant women. The 1,25 dihydroxyvitamin D3 level is higher in pregnant than non-pregnant women. This study aimed to investigate the 1,25 di-hydroxyvitamin D_3 status in vitamin D deficient pregnant women at the first trimester.

Materials and Methods: This study was conducted during December 2011 and April 2012 in 2 hospitals of Istanbul, Turkey. Pregnant women who attended for the regular examination on the 12th gestational week were randomly assigned. They were divided into 2 groups including vitamin D deficient (vitamin D <30 ng/mL) and vitamin D optimal (vitamin D ≥30 ng/mL). Serum 25 hydroxyvitamin D3, 1,25 dihydroxyvitamin D3, fibroblast growth factor 23, calcium, phosphate, urinary calcium, and urinary phosphorus levels were measured.

Results: After exclusion of fourteen pregnant women due to inconvenient serum samples, the mean serum 25 hydroxyvitamin D_3 level of one hundred sixty outpatient pregnant women was 10.8 ± 16.2 ng/mL. Totally, 132 (85.0%) of these women were vitamin D deficient (25 hy-droxyvitamin D_3 level less than 30 ng/mL). Serum 1,25 dihydroxyvitamin D3 and urinary calcium levels were higher in vitamin D deficient than vitamin D optimal group.

Conclusions: Even under severe vitamin D deficiency, the 1,25 dihydroxyvitamin D3 level was high in pregnant women. Active vitamin D status was not compatible with the 25 hydroxyvitamin D_3 level in vitamin D deficient pregnant women at the first trimester.

Keywords: Pregnancy, Vitamin D deficiency, The 1,25 dihydroxyvitamin D3 status

Introduction

Vitamin D is a hormone mostly synthesized in the skin. It regulates calcium homeostasis and bone metabolism. Optimal serum vitamin D levels vary in many reports. In the present study, vitamin D levels were considered as follows: (1) under 9 ng/mL as severe vitamin D deficiency; (2) between 10-19 ng/mL as mild to moderate vitamin D deficiency; (3) between 20-29 ng/mL as vitamin D insufficiency; (4) between 30-149 ng/mL as optimal; and finally, (5) more than 150 ng/mL as intoxication (1,2).

Vitamin D deficiency is associated with a number of signs and symptoms such as osteomalacia, bone pain, weakness, and changes in immune response, renin synthesis, insulin production, and myocardial contractility (1). It is prevalent in pregnant women as well as non-pregnant young adult women. In a recent study, mean vitamin D level of non-pregnant young adult women was 21 ng/mL. In another study, 81.7% of mothers were found vitamin D deficient (vitamin D levels < 25 ng/dL) during delivery (3).

The 1,25 dihydroxyvitamin D3 [$1,25(OH)_2D_3$] is an active form of 25 hydroxyvitamin D3 [$25(OH)D_3$]. The $25(OH)D_3$ is converted to $1,25(OH)_2D_3$ via 1-α hydroxylase enzyme in the kidney. Serum level of $1,25(OH)_2D_3$ is regulated by serum calcium, parathormone (PTH), and fibroblast growth factor 23 (FGF23). Although $1,25(OH)_2D_3$ is the active form of $25(OH)D_3$, the easiest and accurate marker of vitamin D status is serum $25(OH)D_3$ level.

Calcium requirement increases during pregnancy. At the first and second trimesters, $1,25(OH)_2D_3$ levels are much higher than the level at the third trimester. Kidneys are the main sources of $1,25(OH)_2D_3$ in non-pregnant women. However, in pregnant women, the placenta is an additional source of $1,25(OH)_2D_3$. It has vitamin D receptors, 1α-hydroxylase, and 24-hydroxylase enzyme activities (4). After the delivery, $1,25(OH)_2D_3$ level drops immediately (5). A recent meta-analysis (6) showed that $25(OH)D_3$ level was poorly correlated with $1,25(OH)_2D_3$ level in pregnant women. Serum $1,25(OH)_2D_3$ level was high while mean $25(OH)D_3$ level was lower than 10 ng/mL (25 nmol/L). Moreover, the $25(OH)D_3$ level was above 32 ng/mL (80 nmol/L) while $1,25(OH)_2D_3$ level was low

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at term pregnant women. Meanwhile, the $1,25(OH)_2D_3$ status in pregnant women is not clear at the first trimester. Accordingly, in this study, it was aimed to investigate $1,25(OH)_2D_3$ status in pregnant women at the first trimester.

Materials and Methods

The first trimester pregnant women who referred to the outpatient clinic in the Obstetrics and Gynecology Clinics of the Haydarpasa Training Hospital, Gulhane Military Medical Academy (GMMA HTH), and the Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital in Istanbul, Turkey during November 2011 and April 2012 were included in this study. Numbers of birth at the GMMA HTH and the Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital were 543 and 10 005, respectively in 2011. Pregnant women who accepted to participate in the study delivered a written informed consent. Inclusion criteria were body mass index (BMI) <30 kg/m^2 and pregnancy in the first trimester. Obese individuals were excluded because of the adipose tissue stores vitamin D, and thus circulating $25(OH)D_3$ level drops (7). In addition, pregnant women who were on any medication like anticonvulsants or vitamin D supplements, with diabetes mellitus, acute or chronic infections, malabsorption, chronic liver or kidney disease, and underwent surgery for gastrointestinal tract disease were excluded. The pregnant women were classified into 2 groups: the first group included vitamin D deficient women (serum vitamin D levels <30 ng/mL) and the second one contained vitamin D optimal women (serum vitamin D levels ≥30 ng/mL).

Venous blood samples were collected with a dark Vacutainer system (VACUETTE®) after overnight fasting. Then, blood samples were centrifuged for 10 minutes at a low speed (500 x g) and serum samples were stored at -80˚C. Serum $25(OH)D_3$, $1,25(OH)_2D_3$ and FGF23 levels were measured in our biochemistry laboratory. Besides, the FGF23, $25(OH)D_3$ and $1,25(OH)_2D_3$ levels were measured by enzyme-linked immunosorbent assay (ELISA) method using the kits (I) FGF23 (pg/mL, cusabio Biotech, Wuhan, PRChina) (Cat. No: CSB-E10113h, Lot: Y05114515), (II) 25 - hydroxyvitamin D3 (ng/mL, Immundiagnostik AG, Bensheim, Australia) (K2110, Lot: K2110-111 130), and (III) 1,25 dihydroxyvitamin D3 (pg/mL, cusabio Biotech, Wuhan, PRChina) (Cat No. CSB-E05120h, Lot: X07114517). The data obtained by ELISA method were converted to the standard results using a microplate reader RT-2100C (Hamburg, Germany) device.

Routine clinical biochemistry tests were performed on the Abbott Architect (c16000 IL, USA) and Abbott Architect (c16000, IL, USA i2000) auto-analyzers at the Department of Biochemistry, GMMA HTH and with the Roche Cobas Integra (800 Basel, Switzerland) auto-analyzer at the Department of Biochemistry of the Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital.

The distribution of variables was evaluated using the Kolmogorov-Smirnov test. All the data were expressed as mean ± standard deviation (SD) and percentage (%). Furthermore, non-parametric tests were used for abnormally distributed data. Independent groups were compared employing the Mann-Whitney U test. All the statistics were performed at 95% CI. Statistical significance was considered at $P < 0.05$. Moreover, statistical analyses were performed using a PC-compatible statistics program, namely, SPSS software, version 20.0.

Results

One hundred seventy-four pregnant women gave written informed consent. Fourteen pregnant women were excluded due to inappropriate serum samples. Levels of $25(OH)D_3$ and $1,25(OH)_2D_3$ were measured in serum samples of 160 pregnant women. Mean age and mean BMI of the participants were 28.5 ± 5.3 years and 24.4 ± 3.9 kg/m^2, respectively. Besides, the mean of $25(OH)D_3$ level for pregnant women was 10.8 ± 16.2 ng/mL (Table 1). A total of 131 (85.0%) pregnant women had $25(OH)D_3$ levels less than 30 ng/mL.

In addition, the mean of $25(OH)D_3$ level was 5.1 ± 6.7 ng/mL in vitamin D deficient group. This level was classified as severe vitamin D deficiency. In vitamin D optimal group, mean of $25(OH)D_3$ level was 50.7 ± 34.1 ng/mL. The $1,25(OH)_2D_3$ and FGF-23 levels were not significantly different between the vitamin D deficient and optimal groups (Table 2).

Vitamin D deficient group were found to have a lower serum mean calcium level (9.4 ± 0.4 mg/dL) than vitamin

Table 1. Mean Values of Age, BMI, and Biochemical Findings of 160 Pregnant Women

Variable	Mean ± SD
Age (y)	28.5 ± 5.3
BMI (kg/m^2)	24.4 ± 3.9
$25-(OH)D_3$ (ng/mL)	10.8 ± 16.2
$1,25-(OH)_2D_3$ (pg/mL)	6.5 ± 3.0
FGF23 (pg/mL)	5.8 ± 3.2
Serum calcium (mg/dL)	9.4 ± 0.5
Serum phosphate (mg/dL)	3.5 ± 0.5
Serum chlorine (mmol/L)	103.2 ± 1.7
Albumin (g/dL)	4.3 ± 0.3
Urinary calcium (mg/dL)	18.9 ± 16.1
Urinary phosphate (meq/dL)	29.2 ± 24.8
Fasting blood glucose (mg/dL)	87.1 ± 12.5
Blood urea nitrogen (mg/dL)	7.5 ± 1.9
Creatinine (mg/dL)	0.5 ± 0.08

BMI: Body mass index; SD: Standard deviation; FGF23: Fibroblast growth factor 23.

Table 2. Comparison Between Vitamin D Deficient and Optimal Groups

Variable	Vitamin D Deficient Group (Vitamin D <30 ng/mL) (n = 136, 85.0%)	Vitamin D Optimal Group (Vitamin D >30 ng/mL) (n = 24, 15.0%)	P Value
Age (y)	28.3 ± 5.2	29.7 ± 5.4	0.13*
BMI (kg/m^2)	24.5 ± 3.6	23.8 ± 4.5	0.25*
25-(OH)D_3 (ng/mL)	5.1 ± 6.7	50.7 ± 34.1	0.001*
1,25-$(OH)_2D_3$ (pg/mL)	6.6 ± 2.9	6.3 ± 3.4	0.58*
FGF23 (pg/mL)	5.9 ± 3.3	4.9 ± 2.4	0.31*
Serum calcium (mg/dL)	9.4 ± 0.4	9.5 ± 0.6	0.40*
Serum phosphate (mg/dL)	3.5 ± 0.5	3.5 ± 0.5	0.80*
Albumin (g/dL)	4.3 ± 0.3	4.3 ± 0.4	0.78*
Urinary calcium (mg/dL)	18.7 ± 14.6	16.4 ± 12.7	0.42*
Urinary phosphate (meq/dL)	38.6 ± 21.5	41.0 ± 28.5	0.96*
Fasting blood glucose (mg/dL)	87.7 ± 12.0	86.6 ± 9.3	0.76*
Blood urea nitrogen (mg/dL)	7.5 ± 1.9	7.4 ± 1.7	0.85*
Creatinine (mg/dL)	0.5 ± 0.08	0.5 ± 0.09	0.43*

BMI: Body mass index; SD: Standard deviation; FGF23: Fibroblast growth factor 23.
* Mann-Whitney U test.

D optimal group (9.5 ± 0.6 mg/dL) ($P = 0.4$). Although the serum calcium level was lower, urinary calcium level was higher in vitamin D deficient group (18.7 ± 14.6 mg/dL) as compared to that of the vitamin D optimal group (16.4 ± 12.7 mg/dL) ($P = 0.4$).

Base on the results, serum calcium, serum phosphate, urinary calcium, and urinary phosphate concentrations were similar between both groups. However, blood urea nitrogen, serum creatinine, and albumin levels were not statistically significant between the 2 groups.

Discussion

To the best knowledge of the researchers, this is the first study that evaluates $1,25(OH)_2D_3$ level in vitamin D deficient pregnant women at the first trimester. Vitamin D deficiency was prevalent in our cohort. About 85% of the pregnant women (n = 160) had vitamin D levels <30 ng/mL. Mean $25(OH)D_3$ level of vitamin D deficient group was 5.1 ± 6.7 ng/mL which shows severe vitamin D deficiency. This finding correlates with the result of a recent study in which 741 pregnant women (70.6%) had serum vitamin D level less than 10 ng/mL (8).

The $1,25(OH)_2D_3$ level was affected by serum calcium, serum phosphate, serum PTH, and serum $25(OH)D_3$ level. Low $25(OH)D_3$ level causes secondary hyperparathyroidism. Elevated PTH induces 1α-hydroxylase enzyme and converts $25(OH)D_3$ to $1,25(OH)_2D_3$ till severe vitamin D deficiency develops. In severe vitamin D deficiency, tubular calcium reabsorption is increased to correct serum calcium levels (9). A meta-analysis indicated that pregnant women had higher serum $1,25(OH)_2D_3$ level than non-pregnant women and this level showed a linear regression with $25(OH)D_3$ level (6). While $25(OH)D_3$ level was decreasing, $1,25(OH)_2D_3$ level elevated to some degree. In the present study, it was found that $1,25(OH)_2D_3$ level was higher in the vitamin D deficient group than the optimal group but the difference was not statistically significant. This may be explained in 2 ways. First, severe vitamin D deficiency causes secondary hyperparathyroidism and increased 1α-hydroxylase activity that converts $25(OH)D_3$ to $1,25(OH)_2D_3$ leading to a decline in $25(OH)D_3$ level. Second, increased serum $1,25(OH)_2D_3$ begins to induce 24 hydroxylase enzyme and increases catabolism of $25(OH)D_3$ not only in the intestine, kidney, and bone but also in the placenta (4).

A recent study found that low levels of $25(OH)D_3$ and $1,25(OH)_2D_3$ were associated with preterm birth (10).

Based on the results of the current study, serum calcium, serum phosphate, urinary calcium, and urinary phosphate levels were in normal ranges even in severe vitamin D deficiency. These parameters were comparable between the 2 groups. Vitamin D may have less contribution to calcium reabsorption in pregnant women than non-pregnant women. During the pregnancy, other hormones such as human placental lactogen, estrogens, and cortisol are also involved in calcium metabolism (11).

Although $1,25(OH)_2D_3$ is the active form of $25(OH)D_3$, vitamin D status of the body should be estimated by the $25(OH)D_3$ level. The $25(OH)D_3$ level is the best indicator of overall vitamin D status due to its long biological half-life. It also reflects vitamin D from sunlight exposure and dietary intake. The findings of this study showed that $1,25(OH)_2D_3$ level elevated in vitamin D deficient pregnant women at the first trimester. This is compatible with the fact that the correlation between $1,25(OH)_2D_3$ and $25(OH)D_3$ is low in pregnant women at term and

non-pregnant subjects with vitamin D deficiency.

Vitamin D has a role not only in calcium metabolism but also in other systems. The $1,25(OH)_2D_3$ increases insulin production and reduces blood pressure (in short term), plasma renin, and aldosterone levels (12-14). Some studies have shown that vitamin D deficiency might cause poor pregnancy outcomes (15). Risks of small-for-gestational-age infants, preeclampsia, and gestational diabetes have been found to be higher in vitamin D deficiency. In addition, vitamin D deficiency causes preterm birth associated with inflammation and infection (10). Pregnant women with vitamin D deficiency need to be investigated to find that whether the complications are correlated with the active or inactive form of vitamin D3.

In every human attempt, no doubt, there exist some limitations and problems which need to be acknowledged.

No data exist regarding the daily calcium intake of the participants. Daily calcium intake from dietary and supplements affects calcium absorption. A seasonal difference may cause a potential selection bias. Vitamin D levels can be affected by seasonal variation as well. Endogenous vitamin D production via solar UV-B radiation and exogenous vitamin D intake are 2 major sources of vitamin D supply. Because of insufficient sunlight exposure, vitamin D deficiency might be common during winter.

In addition, PTH level was not measured at the beginning of the study due to the study design. Therefore, if we measured PTH levels, vitamin D deficiency might be confirmed by secondary hyperparathyroidism. Besides, non-pregnant women were not included in the study. Vitamin D deficiency is common among non-pregnant women as well. Therefore, the results of the study may not be generalized to other populations. As a result, similar studies focusing on larger sample sizes including non-pregnant women are subject to further investigation.

Conclusions

In general, even under severe vitamin D deficiency, $1,25(OH)_2D_3$ level was stable in pregnant women at the first trimester. The $1,25(OH)_2D_3$ level was not correlated with FGF23 level in vitamin D deficient pregnant women. It is hard to predict $1,25(OH)_2D_3$ status with the measurement of serum $25(OH)D_3$ level in pregnant women. Accordingly, more studies should be conducted to understand $1,25(OH)_2D_3$ status during the pregnancy.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

This cross-sectional study was approved by the local Ethics Committee of the Gulhane Military Medical Academy in October 31, 2011 and conducted based on the Helsinki Declaration.

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References

1. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281. doi:10.1056/NEJMra070553
2. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc. 2010;85(8):752-757; quiz 757-758. doi:10.4065/mcp.2010.0138
3. Ergur AT, Berberoglu M, Atasay B, et al. Vitamin D deficiency in Turkish mothers and their neonates and in women of reproductive age. J Clin Res Pediatr Endocrinol. 2009;1(6):266-269. doi:10.4274/jcrpe.v1i6.266
4. Zehnder D, Evans KN, Kilby MD, et al. The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua. Am J Pathol. 2002;161(1):105-114. doi:10.1016/s0002-9440(10)64162-4
5. Ritchie LD, Fung EB, Halloran BP, et al. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr. 1998;67(4):693-701. doi:10.1093/ajcn/67.4.693
6. Papapetrou PD. The interrelationship of serum 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in pregnancy at term: a meta-analysis. Hormones (Athens). 2010;9(2):136-144.
7. Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med. 2013;10(2):e1001383. doi:10.1371/journal.pmed.1001383
8. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2008;24(1):27-32. doi:10.1002/dmrr.737
9. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116(8):2062-2072. doi:10.1172/jci29449
10. Thota C, Menon R, Fortunato SJ, Brou L, Lee JE, Al-Hendy A. 1,25-Dihydroxyvitamin D deficiency is associated with preterm birth in African American and Caucasian women. Reprod Sci. 2014;21(2):244-250. doi:10.1177/1933719113493513
11. Kovacs CS. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr. 2008;88(2):520s-528s. doi:10.1093/ajcn/88.2.520S
12. Carrara D, Bernini M, Bacca A, et al. Cholecalciferol administration blunts the systemic renin-angiotensin system in essential hypertensives with hypovitaminosis D. J Renin Angiotensin Aldosterone Syst. 2014;15(1):82-87.

doi:10.1177/1470320312471149
13. Judd SE, Raiser SN, Kumari M, Tangpricha V. 1,25-dihydroxyvitamin D3 reduces systolic blood pressure in hypertensive adults: a pilot feasibility study. J Steroid Biochem Mol Biol. 2010;121(1-2):445-447. doi:10.1016/j.jsbmb.2010.04.013
14. Kayaniyil S, Retnakaran R, Harris SB, et al. Prospective associations of vitamin D with beta-cell function and glycemia: the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study. Diabetes. 2011;60(11):2947-2953. doi:10.2337/db11-0465
15. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ. 2013;346:f1169.