

Research Article

Is 1,25 Dihydroxy Vitamin D Measurement Useful for the Diagnosis of Vitamin D Deficiency in Early Pregnancy?

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Abstract

Objectives: 1,25 dihydroxyvitamin D [1,25(OH)₂D] starts to rise from the first trimester of pregnancy and increases maternal intestinal calcium absorption. The preferred method in the diagnosis of Vitamin D deficiency is the measurement of 25 hydroxyvitamin D [25(OH)D]. However, fetal and placental structures produce 1,25(OH)₂D and affect calcium metabolism during pregnancy. In this study, we aimed to evaluate 25(OH)D and 1,25(OH)₂D levels in early pregnancy.

Methods: Thirty-eight pregnant and 28 non-pregnant women were included in the study. Their 25(OH)D, 1,25(OH)₂D, calcium, albumin, and parathormone levels were evaluated.

Results: 25(OH)D levels were below 20 ng/mL in both groups, but lower in the pregnant group (mean 12.3 [±7.5] ng/mL in pregnant, and 19.4 [±14.4] ng/mL in non-pregnant, p=0.017). 1,25(OH)₂D levels were higher in the pregnant group (mean 105.9 [±29.2] pg/mL in pregnant, and 81.5 [±31.9] pg/mL in non-pregnant, p=0.003).

Conclusion: 1,25(OH)₂D plays a primary role in meeting the increased calcium need during pregnancy and provides a protective effect in terms of both fetal and maternal skeletal complications. The increase in 1,25(OH)₂D starting from the first trimester is a pregnancy-specific mechanism in enhancing intestinal calcium absorption and providing the calcium requirement for the developing fetus. Thus, it may be useful to measure 1,25(OH)₂D, in addition to 25(OH)D, when deciding on Vitamin D replacement in pregnant women of any trimester.

Keywords: 1, 25 dihydroxy vitamin D, 25 hydroxyvitamin D, first trimester, pregnancy, Vitamin D

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The fetus's need for calcium is provided by the mother during pregnancy. The major source is intestinal calcium absorption, which increases 2–3 times, in pregnant women.^[1] Parathormone (PTH) and Vitamin D play important roles in maintaining calcium balance in the body. PTH levels do not change significantly during pregnancy. The increase in intestinal calcium absorption is provided by calcitriol, the active form of Vitamin D.^[2]

Vitamin D is a steroid hormone. Whether it is taken with food or synthesized in the skin, it is in inactive form and

is converted to its active metabolites, 25-hydroxyvitamin D [25(OH)D] and 1,25 dihydroxyvitamin D [1,25(OH)₂D], in the liver and the kidneys, respectively. These metabolites, also called calcidiol and calcitriol, play an important role in calcium homeostasis and bone metabolism. The circulating amount of 25(OH)D is measured in ng/mL and has a half-life of 2–3 weeks.^[3] 1,25(OH)₂D is found in the circulation in lesser amounts (at pg/mL levels) than 25(OH)D, but it is more potent. 1,25(OH)₂D regulates gene transcription by binding to intracellular receptors in target tissues.

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1,25(OH)₂D increases intestinal calcium absorption, decreases renal calcium excretion, and mobilizes calcium to the circulation from bones.

Production of 1,25(OH)₂D is regulated by blood PTH, calcium, and phosphorus levels and inhibited by bone-derived fibroblast growth factor-23 (FGF-23).^[4] Measurement of 25(OH)D level is preferred in the diagnosis of Vitamin D deficiency, because of its longer half-life. In addition, the fact that the production of 25(OH)D in the liver is primarily dependent on the presence of substrate and is not regulated by other mechanisms such as 1,25(OH)₂D, provides more reliable results.^[3] As a consensus, serum 25(OH)D levels below 20 ng/mL are considered as Vitamin D deficiency and adversely affect bone health.^[5, 6]

With the detection of Vitamin D receptor in many tissues and cells in the body, interest in the relationship of this vitamin with various diseases and conditions has risen. Low Vitamin D levels are found to be associated with malignancies, autoimmune diseases, cardiovascular diseases, and some infections in epidemiological studies.^[4, 7-9] Some studies in pregnant women have shown that Vitamin D deficiency may be associated with pregnancy complications such as pre-eclampsia.^[10, 11] It has been suggested that both developmental and calcium metabolism-related complications (such as intrauterine growth retardation, pre-term birth, stillbirth, neonatal hypocalcemic seizure, and postnatal growth retardation) may occur in the fetus, due to insufficient nutritional and mineral content in the intrauterine environment at important stages of fetal development, as a result of maternal Vitamin D deficiency.^[12-17]

The serum 1,25(OH)₂D levels in pregnant women increase approximately 2 times. It is both due to the increase in Vitamin D binding globulins with the effect of estrogen and with the increase in maternal renal 1- α hydroxylase and placental 1- α hydroxylase activities.^[1] The raise in 1,25(OH)₂D increases intestinal calcium absorption and plays an active role in meeting the increasing fetal calcium requirement as pregnancy progresses. A total of 25–30 g of calcium is transferred from the mother to the fetus during pregnancy.^[18] The increase in 1,25(OH)₂D continues throughout pregnancy to meet fetal calcium needs and to maintain normal maternal serum calcium homeostasis.^[19]

In this study, the 25(OH)D and 1,25(OH)₂D levels of women in the first trimester of pregnancy, who do not use any medication, were compared with the non-pregnant control group of similar age.

Methods

The 25(OH)D, 1,25(OH)₂D, calcium, albumin, and PTH levels of the patients, who applied to the outpatient clinic in the

first trimester of pregnancy, were measured and compared with those of the age and gender-matched control group. Thirty-eight pregnant and 28 non-pregnant women were included in the study. Exclusion criteria were the presence of liver or kidney failure and any drug use, in both groups. To minimize the effects of seasonal changes, those who applied in July and August, instead of the winter season when Vitamin D levels tend to be low, were included in the study. 25(OH)D and 1,25(OH)₂D were measured by the chemiluminescence microparticle immunoassay method and the chemiluminescent immunoassay method, respectively.

All participants' written informed consents have been obtained and the study was approved by the Eskisehir Osmangazi University Non-interventional Clinical Research Ethics Committee (Decision No: 07, Date: 19.12.2017).

Statistical Analysis

Continuous data are given as mean \pm standard deviation. Shapiro–Wilk's test was used to investigate the appropriateness of the data to normal distribution. Provided that the data has a normal distribution, independent sample t-test analysis was used to compare two groups. The comparison of the groups that do not fit normal distribution was performed using the Mann–Whitney U-test. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, v. 21.0. Armonk, NY: IBM Corp.) was used in the implementation of the analyses. $P < 0.05$ was considered as a criterion for statistical significance.

Results

There was no statistically significant difference between the pregnant women and the control group in terms of calcium, phosphorus, and PTH levels. 25(OH)D levels were below 20 ng/mL in both groups, but lower in the pregnant group (mean 12.3 [\pm 7.5] ng/mL in the pregnant group, and 19.4 [\pm 14.4] ng/mL in the control group, $p=0.017$). The mean levels of 1,25(OH)₂D were within the reference ranges in both groups, but the pregnant group's mean was higher, with statistical significance (mean 105.9 [\pm 29.2] pg/mL in the pregnant group, and 81.5 [\pm 31.9] pg/mL in the control group, $p=0.003$). Mean age, calcium, phosphorus, PTH, and Vitamin D levels of the patients are shown in Table 1.

Discussion

In our study, the mean 25(OH)D levels of both groups were found to be below 20 ng/mL, consistent with Vitamin D deficiency, although they were examined in the summer months. The 25(OH)D levels of the pregnant women were found to be significantly lower than the control group. As

Table 1. Mean age, calcium, phosphorus, parathormone, and Vitamin D levels of the patients

	Pregnant (n=34)	Control (n=28)	Reference range	p
Age	27.6±5.3	24.9±5.2	–	>0.05
Calcium (mg/dl)	9.6±0.4	9.6±0.4	8.8–10.6	>0.05
Phosphorus (mg/dl)	3.3±0.5	3.6±0.6	2.5–4.5	>0.05
Parathormone (pg/mL)	38.8±24.2	47.0±17.1	15–68.3	>0.05
25(OH)D (ng/mL)	12.3±7.5	19.4±14.4	6.5–50	0.017
1,25(OH) ₂ D (pg/mL)	105.9±29.2	81.5±31.9	26.1–95	0.003

25(OH)D: of 25 hydroxyvitamin D, 1,25(OH)₂D: 1,25 dihydroxyvitamin D

expected, the 1,25(OH)₂D levels of the pregnant women were significantly higher than the control group. PTH and calcium levels of both groups were within the normal range. These findings suggest that even if 25(OH)D is low, the increase in 1,25(OH)₂D levels during pregnancy stabilizes the calcium and PTH levels of the pregnant woman. However, it is difficult to predict whether 1,25(OH)₂D increase alone will be sufficient to prevent potential negative effects (such as pre-term birth and low birth weight) that may arise from low 25(OH)D levels in the later months of pregnancy and to protect the mother's bone health.

Vitamin D and its metabolites are transported in the circulation by binding primarily to Vitamin D binding protein (DBP) and a lesser extent to albumin and lipoproteins; <1% circulates in the unbound (free) form.^[20] In some special conditions where DBP levels change (e.g., liver cirrhosis or use of oral contraceptives), free 25(OH)D measurements can be used. Although DBP increases during pregnancy, free 25(OH)D has been shown to increase as well, and it has been associated with a decrease in DBP affinity. Therefore, more data are needed to demonstrate the clinical significance of free 25(OH) D measurement in pregnancy.^[20, 21] Total 1,25(OH)₂D levels are also elevated due to increased binding globulins during pregnancy. Independent of this increase and free 1,25(OH)₂D has also been shown to be elevated due to the increase in its production.^[22] The lack of free 25(OH)D and free 1,25(OH)₂D measurements is a limitation of our study.

While PTH is at a low-normal level in the first trimester of pregnancy, it increases to the mid-normal range toward the term. The increase in 1,25(OH)₂D occurs due to the increase in the activity of maternal renal 1- α hydroxylase independent of PTH. Furthermore, the placenta, with its 1- α hydroxylase activity, and the decidua and fetal kidneys, in addition, are thought to contribute to the production of 1,25(OH)₂D at low amounts.^[23–25] The increase in 1- α hydroxylase activity is mediated by PTH-related protein (PTHrP), estradiol, prolactin, and placental lactogen, whose serum concentrations increase during pregnancy. PTHrP is

produced by many tissues and high levels in pregnancy are thought to originate from breast tissue, decidua, amnion, and placenta; and it is thought to play a role in calcium transport to these tissues.^[2]

In healthy women, the mechanisms that are effective in meeting the fetus's calcium need during pregnancy and breastfeeding do not cause significant permanent damage to the mother's bone structure when these periods are over.^[18, 23] However, in countries with low socioeconomic status, considering the multiparity, it can be predicted that women with nutritional deficiencies are at higher risk for skeletal complications after pregnancy and lactation.^[26] Women living in northern latitudes may also be at risk, as they may not get enough sunlight. Today, there are different opinions about monitoring Vitamin D levels and the treatment of deficiency in pregnant women.^[7] Measurement of Vitamin D in the early stages of pregnancy in high-risk populations is important in terms of providing the chance to protect the mother and the baby from the negative consequences that may occur by intervening promptly on time. However, evaluating 25(OH)D alone may not provide sufficient information about Vitamin D status. Because of the additional units such as placenta, fetal structures, and breast tissue that play roles in the regulation of calcium metabolism, and the adaptive mechanisms that occur with their effects during pregnancy, evaluation of Vitamin D should also differ from non-pregnant women. Considering that the increase in 1,25(OH)₂D starting from the first trimester is a pregnancy-specific mechanism in enhancing the intestinal calcium absorption and providing calcium for the developing fetus, we think that it may be useful to measure 1,25(OH)₂D, in addition to 25(OH)D, when deciding on Vitamin D replacement.

Our study is important for the reason that it showed that 1,25(OH)₂D is increased and calcium and PTH levels are normal in pregnant women, who were thought to get replacement therapy according to 25(OH)D levels, even though it was measured especially in the time when the benefit from sunlight is high. However, studies evaluating active Vitamin

D and its long-term results in larger patient populations are needed to determine the required 1,25(OH)₂D levels during pregnancy and the cut-point levels at the start of treatment.

Conclusion

The increase of 1,25(OH)₂D, which starts from the first trimester and continues until the term, plays a primary role in meeting the increased calcium need during pregnancy and provides a protective effect in terms of both fetal and maternal skeletal complications. Therefore, considering the 1,25(OH)₂D levels may contribute to the decision when initiating Vitamin D replacement therapy in pregnant women.

Disclosures

Ethics Committee Approval: All participants' written informed consents have been obtained and the study was approved by the Eskisehir Osmangazi University Non-interventional Clinical Research Ethics Committee (Decision No: 07, Date: 19.12.2017).

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