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ASSESSMENT OF CORRELATION BETWEEN VITAMIN D LEVEL AND PREVALENCE OF PRETERM BIRTHS IN THE POPULATION OF PREGNANT WOMEN IN POLAND

MARTA BACZYŃSKA-STRZECHA and JAROSŁAW KALINKA

Medical University in Lodz, Łódź, Poland
Department of Perinatology, 1st Chair of Obstetrics and Gynecology

Abstract
Objectives: Aim of this project is determination of the correlation between the level of vitamin D in blood serum and duration of pregnancy in population in central Poland. **Material and Methods:** 25-Hydroxyvitamin D (25(OH)D) level was determined in blood serum, using enzyme-linked immunosorbent assay (ELISA). Standardized history of each patient was recorded. The history included: general medical history, data regarding the course of pregnancy and information about health-related behavior that could influence vitamin D concentration. Two hundred-and-one Caucasian women at childbirth were qualified into the study. The study group was divided into 2 parts: 100 patients who had a spontaneous premature birth and 101 patients who had birth at full term. **Results:** Vitamin D deficiency (< 30 ng/ml) was very common for both groups (69.6% of patients in the premature group and 72% – in the control group). Patients who had a premature birth had severe vitamin D deficiency (less than 10 ng/ml) more often than in the control group (34% vs. 14.2%, p = 0.001). Severe vitamin D deficiency increased the risk of premature birth but the association was not statistically significant in the multivariate regression model (odds ratio (OR) = 2.47, 95% confidence interval (CI): 0.86–7.15, p = 0.094). **Conclusions:** Severe vitamin D deficiency (< 10 ng/ml) may be the factor increasing the risk of preterm birth. Int J Occup Med Environ Health 2017;30(6)

Key words:
Pregnancy, Vitamin D, Preterm delivery, Vitamin D deficiency, Preterm birth, Preterm labor

INTRODUCTION

Preterm births are currently the main cause of perinatal mortality and morbidity of neonates, and have a significant influence on their further mental and physical development. Prematurity is not only a huge medical problem but also a social and economic one [1].

There are studies suggesting that vitamin D deficiency in the case of pregnant women and neonates is common in Poland, and associated with poor exposition to sunlight at that geographical latitude (49–55°N) and low dietary consumption of the vitamin by pregnant women [2]. Furthermore, it has recently been considered that abnormal gestational vitamin D level (< 30 ng/ml) has an unfavorable effect both on mother and a fetus [3,4].

Only single reports indicating the effect of vitamin D on duration of pregnancy have been published so far.

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Corresponding author: M. Baczyńska-Strzecha, Medical University in Lodz, Department of Perinatology, 1st Chair of Obstetrics and Gynecology, Wileńska 37, 94-029 Łódź, Poland (e-mail: m.baczynska@onet.eu).

The role of the vitamin is explained mostly by its participation in immunological processes and its role in modulation of bacterial infection [5–8].

Determination of the optimum gestational vitamin D level and of the role of vitamin D in pregnancy is of utter importance in terms of the ongoing discussion of the optimum vitamin D supplementation for pregnant women. Guidelines published in 2013 and based on the review of literature on supplementation of vitamin D and treatment of deficits of the vitamin in Central Europe recommend supplementation of pregnant women with a dose of 1500–2000 IU/day from the 2nd trimester of pregnancy, and consideration of supplementation introduction from the very beginning of pregnancy in the case of patients demonstrating abnormal serum vitamin D level (< 30 ng/ml) [9]. On the other hand, the World Health Organization (WHO) recommendations issued in 2012 indicate that vitamin D supplementation during pregnancy is not necessary because of the lack of clear evidence of its efficacy in treatment of pregnancy pathologies [10].

The purpose of this study has been to determine the correlation between the vitamin D level in blood serum and duration of pregnancy in population in central Poland.

MATERIAL AND METHODS

The study was carried out in 2013–2015 at the Department of Perinatology, 1st Chair of Gynecology and Obstetrics, Medical University in Lodz, Poland. Patients hospitalized at the Center were inhabitants of the central macro-region of Poland, and formed an ethnically homogenous population.

The study was approved by the Bioethics Committee of the Medical University in Lodz, Poland, authorization No. RNN/74/12/KE, and all participants gave their informed consents on participation in the study. A detailed, standardized medical history was obtained from all patients. The questionnaire included questions regarding demographic, medical and obstetric issues.

This was a case-control study. Caucasian women, of Polish origin, in single pregnancy, giving birth at the Department of Perinatology were qualified into the study. Patients were divided into 2 groups. The test group involved 100 women having spontaneous preterm birth (weeks 22–36.6). The control group involved 101 women having term birth (> 37 weeks). Duration of pregnancy was determined based on the date of the last menstruation, and verified with an ultrasonographic examination performed between week 11 and 13.6 of pregnancy. All patients were included in the study on the day of childbirth after triggering the regular uterine systolic action.

Exclusion criteria were: multiple pregnancy, abnormal result of prenatal screening, anatomical or genetic defects of the fetus, iatrogenic preterm delivery, pre-pregnancy diabetes, severe arterial hypertension present before the pregnancy or pregnancy-induced (blood pressure above 160/110 mm Hg), immunological disorders, maternal severe endocrine diseases (uncompensated hypothyroidism, hyperparathyroidism, parathyroid diseases and adrenal disorders), generalized infection. Additionally, patients with diseases that could affect the vitamin D level – chronic renal failure, condition post surgical procedures of the upper alimentary tract, parathyroid diseases, hepatic problems, uncontrolled thyroid diseases, unspecific inflammatory enteral diseases, were excluded.

Blood was collected from patients on the day of childbirth. 25-Hydroxyvitamin D (25(OH)D) level was assayed at the Immunoendocrinology Department of the Chair of Endocrinology of the Medical University in Lodz. The 25(OH)-Vitamin direct ELISA Kit from Immundiagnostik produced in Germany was used for the assay. 25-Hydroxyvitamin D level was measured in serum obtained from 5 ml of full blood using the immunoenzymatic method. The immunoenzymatic method – enzyme-linked immunosorbent assay (ELISA) consisted in the use of monoclonal antibodies recognizing 25(OH)D. Concentration of vitamin D was determined quantitatively using

a spectrophotometric method, based on the measurement of color intensity. Concentration of 25(OH)D in samples was determined based on the standard curve. Quality control was performed. Standards, controls and patient samples were incubated together. The results for the patient samples were not valid, if within the same assay one or more values of the quality control sample was outside the acceptable limits (4.8–96 ng/ml). Limit of detection was 1.28 ng/ml.

Vitamin D deficiency was classified as less than 30 ng/ml. Obtained results of serum vitamin D levels were divided into 4 categories. Severe vitamin D deficiency (< 10 ng/ml), moderate deficiency (10–20 ng/ml), suboptimal vitamin D level (20–30 ng/ml), and the level over 30 ng/ml was considered normal.

The calculations were performed using Statistica PL 10.0 software. Quantitative variables were described by the mean (M), standard deviation (SD) and range (min.–max). Four categorical variables percentages (%) are presented. Normality was tested using the Shapiro-Wilk's test for normality. Differences between 2 independent samples for continuous data were analyzed using Mann-Whitney U test (if the distributions of variables were different from normal) or Student's t-test (for variables normally distributed). For the categorical variables the statistical analysis was based on Pearson Chi^2 test or Chi^2 test with Yates' adjustment. Variables significant in univariate comparisons (at $p < 0.10$) were included into the multivariate logistic regression model to identify the set of the independent risk factors of preterm delivery. The odds ratio (OR) with 95% confidence interval (CI) was presented for each potential risk factor. The results were considered statistically significant at $p \leq 0.05$.

RESULTS

One hundred patients who had preterm birth and 101 patients who had term birth finally participated in this study. Both groups were comparable in terms of the mean age, mean body mass index (BMI), vocational activity, and living conditions ($p > 0.05$). Selected characteristics are presented in the Table 1.

The analysis of health-related behavior that could influence vitamin D concentration revealed that the ratio of patients using supplementation of vitamin D, most commonly from the 2nd trimester of pregnancy, was high and similar in both groups (78.1% vs. 83.8%, $p = 0.309$). Women giving term birth significantly more often used vitamin preparations containing vitamin D throughout their pregnancy (40.8% vs. 26.9%, $p = 0,042$). Additionally, they significantly more often declared a correct time of daily exposure to sunlight adequate to synthesis of vitamin D (47.5% vs. 25.6%, $p = 0.002$) (Table 1).

Vitamin D deficiency, that is the level below 30 ng/ml, was found very often in the case of both groups, in as many as 69.6% of patients in the control group and as many as 72% in the study group ($p = 0.711$).

The analysis of the mean serum vitamin D level in subgroups demonstrated lower levels in the preterm birth group (22.2±16.9 ng/ml) as compared to the term birth group (23.8±11.1 ng/ml) but the difference was not statistically significant ($p = 0.103$). The minimum level of vitamin D observed in the preterm birth group was 0.32 ng/ml and 1 ng/ml in the term birth group. The maximum level of vitamin D observed in the preterm birth group was 74.6 ng/ml and 41.8 ng/ml in the term birth group.

Women having preterm birth significantly more often had vitamin D level below 10 ng/dl – severe vitamin D deficiency (34% vs. 14.2%, $p = 0.001$). The percentage of patients with normal vitamin D level was higher for the term birth group than for the preterm birth group but the difference was not statistically significant (30.4% vs. 28%, $p = 0.714$). Results are presented in the Table 2.

Additionally, the preliminary analysis revealed that the mean vitamin D level in the group of patients giving birth before the 34th week was only 16.49 ng/ml, and was significantly lower than in the group of, the so called, late pre-

Table 1. Characteristics of the respondents in the study of the correlation between the level of vitamin D in blood serum and duration of pregnancy, Poland, 2013–2015

Characteristics	Respondents		p
	who gave preterm birth (N = 100)	who gave term birth (N = 101)*	
Age [years] (M±SD (min.–max))	31.0±5.0 (19.0–42.0)	29.9±4.5 (19.0–40.0)	0.114
BMI [kg/m^2] (M±SD (min.–max))	26.6±4.6 (18.7–39.3)	27.1±3.3 (18.6–38.8)	0.198
Body weight [kg] (M±SD (min.–max))	71.7±13.9 (46.0–112.0)	74.6±9.5 (55.0–108.0)	0.019
Pregnancies [n] (M±SD (min.–max))	2.0±1.2 (1–7)	1.6±0.8 (1–4)	0.007
Births [n] (M±SD (min.–max))	1.7±1.0 (1–7)	1.4±0.6 (1–4)	0.028
Caesarean section [n (%)]	51 (51.0)	23 (22.8)	< 0.001
Uterine cervix insufficiency [n (%)]	25 (25.0)	4 (4.0)	< 0.001
PROM/PPROM [n (%)]	66 (66.0)	24 (24.0)	< 0.001
Hospitalizations** [n (%)]	64 (64.0)	39 (39.0)	< 0.001
Miscarriages [n (%)]	19 (19.0)	9 (8.9)	0.030
Nicotinism [n (%)]	19 (19.0)	10 (10.1)	0.071
Infections occurrence** [n (%)]	25 (25.0)	15 (14.9)	0.071
Vitamin D supplementation [n (%)]			
during pregnancy	78 (78.0)	83 (83.8)	0.309
throughout pregnancy	26 (26.0)	40 (40.8)	0.042
Appropriate daily exposure to sunlight** [n (%)]	25 (25.0)	47 (47.5)	0.002

* Some percentages are not calculated from N = 101 because of missing data.
** During pregnancy.
M – mean; SD – standard deviation; min. – minimal value; max – maximal value; BMI – body mass index; PROM – premature rupture of membranes; PPROM – preterm premature rupture of membranes.

term deliveries, that was between week 34 and 36.6, where it was 25.05 ng/ml (p = 0.042). Abnormal vitamin D level was found more often before the week 34 (83.8%) than between week 34 and 36.6 (66.2%), however, the difference was not statistically significant (p = 0.116). In this analysis the participants were divided into only 2 subgroups (weeks 22–33.6 and 34–36.6). The number of patients was not big enough for the proper division into 3 subgroups (weeks 22–31.6, 32–33.6 and 34–36.6).

The logistic regression model was applied to determine the effect of the vitamin D level on the risk of preterm delivery. Variables for which statistically significant differences had been found (body weight, number of pregnancies, number of deliveries, history of miscarriage, nicotinism, infections during pregnancy, premature rupture of membranes (PROM), cervical insufficiency during pregnancy) were selected for creation of the logistic regression model. The univariate logistic regression model demonstrated that the level of vitamin D below 10 ng/ml significantly increased the risk of preterm birth (OR = 2.62, 95% CI: 1.15–5.98, p = 0.023). In the multivariate model, besides the level of vitamin D, factors significant in univariate logistic regression model at the level of p < 0.10 were taken into account. Variables affecting the concentration of vitamin D and statistically significant were not taken into account. The analysis indicated 2 statistically significant independent variables increasing the risk of preterm birth: PROM (OR = 7.25, 95% CI: 3.28–16.05, p < 0.001)

Table 2. Vitamin D level in serum of the respondents on the day of childbirth, Poland, 2013–2015*

Vitamin D concentration	Respondents [n (%)]		p
	who gave preterm birth (N = 100)	who gave term birth (N = 92)	
< 10 ng/ml (severe deficiency)	34 (34.0)	13 (14.2)	< 0.001
10–20 ng/ml (moderate deficiency)	17 (17.0)	23 (25.0)	0.17
20–30 ng/ml (suboptimal level)	21 (21.0)	28 (30.4)	0.13
> 30 ng/ml (normal level)	28 (28.0)	28 (30.4)	0.71

* Level of statistical significance was set at $p \leq 0.05$.

Table 3. Logistic regression model* of demographic, behavioral and clinical characteristics associated with preterm delivery, Poland, 2013–2015

Variable	OR (95% CI)	p
Body weight	0.973 (0.942–1.004)	0.091
Pregnancies	1.149 (0.246–5.375)	0.860
Deliveries	1.567 (0.298–8.229)	0.596
Miscarriages	1.075 (0.105–11.026)	0.951
Nicotinism	1.962 (0.588–6.539)	0.273
Infections	2.365 (0.902–6.196)	0.080
Premature rupture of membranes (PROM)	7.571 (3.372–16.998)	0.001
Uterine cervix insufficiency	9.409 (2.523–35.092)	0.001
Vitamin D level		
< 10 ng/ml	2.543 (0.880–7.351)	0.085
10–20 ng/ml	0.367 (0.119–1.125)	0.080
20–30 ng/ml	0.722 (0.269–1.934)	0.517

* $Chi^2 = 69.52$, df = 11, $p < 0.001$ – statistically significant model.
OR – odds ratio; CI – confidence interval.

and cervical insufficiency (OR = 9.21, 95% CI: 2.48–34.19, p = 0.001). The vitamin D level below 10 ng/ml also increased the risk of preterm birth, but the result was not statistically significant (OR = 2.47, 95% CI: 0.86–7.15, p = 0.094) (Table 3).

The analysis of data from the group with severe vitamin D deficiency revealed low percentage of patients supplemented vitamin D in this group (65.9% (< 10 ng/ml) vs. 85.1% (> 10 ng/ml), p = 0,005). Additionally, women taking preparations containing vitamin D at a dose of 800–1000 IU were more frequently in the group of patients giving birth on time (21.2% vs. 15.7%, p = 0.33). Furthermore, maximum duration of daily exposure to the sun was shorter in this group (15±34.5 min vs. 44.8±84.2 min, p = 0.019) and lower percentage of patients declared appropriate to synthesize vitamin D time of daily exposure to sunlight unlike the group with vitamin D level > 10 ng/ml (22.5% vs. 41%, p = 0.028) (Table 4).

Table 4. Characteristics of parturient respondents by vitamin D level in serum, Poland, 2013–2015

Characteristics	Respondents		p
	< 10 ng/ml of vitamin D (N = 47)	> 10 ng/ml of vitamin D (N = 145)	
Age [years] (M±SD (min.–max))	31.0±4.8 (19.0–40.0)	30.3±4.8 (19.0–42.0)	0.414
BMI [kg/m^2] (M±SD (min.–max))	27.6±3.7 (21.0–37.3)	26.5±4.0 (18.6–39.3)	0.129
Body weight [kg] (M±SD (min.–max))	74.5±11.4 (57.0–104.0)	72.4±11.9 (46.0–112.0)	0.320
Maximum duration of daily exposure to the sun* [min] (M±SD (min.–max))	15.0±34.5 (0–120)	44.8±84.2 (0–480)	0.019
Hospitalizations* [n (%)]	28 (63.6)	66 (46.5)	0.047
Vitamin D supplementation [n (%)]			
during pregnancy	29 (65.9)	120 (85.1)	0.005
throughout pregnancy	9 (21.9)	51 (36.4)	0.084
Appropriate daily exposure to sunlight* [n (%)]	9 (22.5)	57 (41.0)	0.028

* During pregnancy.
Abbreviations as in Table 1.

DISCUSSION

In this study we found a significantly higher ratio of patients with severe vitamin D deficiency (< 10 ng/ml) in the group of patients having spontaneous preterm birth as compared to women with term delivery. The model of logistic regression created by us indicates a possible association between severe vitamin D deficiency and occurrence of preterm births (OR = 2.47, p = 0.094); however, the result is not statistically significant and further studies on a larger groups of patients are necessary. In addition, it has been found that the concentration of vitamin D below 15.84 ng/ml significantly increases the risk of preterm birth.

The analysis of healthy-related behavior that may influence vitamin D level also revealed the correlation between vitamin D and preterm birth. In the group of patients who supplemented vitamin D only periodically during their pregnancy, preterm births were more often. Currently, it is recommended to supplement vitamin D in doses of 2000 IU. In the population we analyzed, most patients used supplementation but the doses were below the currently recommended ones. We found that patients who declared avoiding the sun and not adequate time of daily exposure to sunlight also gave birth prematurely. The further analysis of the group of patients with severe vitamin D deficiency also revealed the correlation between vitamin D level and its supplementation or exposure to sunlight.

Additionally, the analysis of vitamin D levels after dividing patients into groups revealed that the mean level of vitamin D was lower in the group of birth before 34 week of gestation (early preterm birth) than in the group of birth between 34–36.6 week of gestation (late preterm birth). It may be concluded that an earlier birth time correlates with a lower vitamin D level.

Another important finding from our study is the fact that vitamin D below optimal level (> 30 ng/ml) is common among the studied population. The study indicated a very high ratio of patients with diagnosed vitamin D deficiency as much as 70% of the whole studied population. We found a high ratio (up to 80%) of patients using vitamin D-containing preparations during their pregnancy. However, the use of

vitamin D-containing preparations throughout pregnancy was declared mostly by patients giving birth on time, and in the study group the ratio was much lower.

Bartoszewicz et al. [2] also found a high percentage of vitamin D deficiency in the population of Polish pregnant women. In their study on a population of 50 healthy pregnant volunteers living in Warszawa, and having term birth, the optimum vitamin D concentration (30–80 ng/ml) was found in only 30% of participants, and a severe deficiency, defined as the vitamin level below 10 ng/ml, was found in 7.3% of studied pregnant women [2]. In our study, severe deficiency was found in a bigger group of patients: as many as 14% of patients having term birth and as many as 34% of patients having preterm birth.

Little is known about maternal vitamin D status in relation to risk of spontaneous preterm birth. There are single reports suggesting an association between vitamin D deficiency and occurrence of preterm births.

Shibata et al. [5] performed their study on a group of pregnant women in 30th week of pregnancy and found a significantly lower vitamin D levels for patients hospitalized because of threatened premature delivery. The vitamin D level in that group was 11.2±3.2 ng/ml and was significantly lower as compared to healthy gravidas, demonstrating the level of 15.6±5.1 ng/ml. The study also demonstrated that pregnant women with abnormal serum vitamin D levels required treatment for threatened premature delivery 0.023-times more often. Authors did not provide any information regarding: efficacy of the treatment for the threatened preterm delivery, how many of the studied patients had preterm birth despite the introduced therapy, and what the vitamin D concentration was in the group of patients in which the treatment proved successful [5].

Bodnar et al. [7] stated that abnormal vitamin D level below 75 nmol/l (30 ng/ml) was associated with the increased risk of preterm delivery in the case of women in twin pregnancy. In that study, blood was collected from all patients at week 24–28 of pregnancy – that is different from our study. The study indicated that the vitamin D level above 30 ng/ml decreased the risk of preterm birth by 60%. The researchers stressed that demand for microelements, including vitamin D, was different in the case of a twin pregnancy and singleton pregnancy, and standards of recommended supplementation should be different as well [7].

In the study by Thorp et al. [6] on pregnant women with a history of at least one preterm delivery, no effect of abnormal vitamin D level, understood as the concentration below 50 nmol/l (20 ng/ml), on duration of pregnancy was found. The study analyzed 131 patients having preterm birth and 134 having term birth, but the study population was not ethnically homogeneous, as much as 40% of patients were Afro-Americans, and 12% were Latin Americans. A similar distribution was found in the control group. In that study blood was collected twice: in week 16–22 and 25–28, but no level on the day of delivery was measured [6]. In our study the population was ethnically homogeneous, and vitamin D level was determined at a different point of pregnancy, which could result in different results.

Maintenance of pregnancy depends on an equilibrium between the anti-inflammatory and the antibacterial response in the maternal-fetal system. Vitamin D plays a role in modulation of both.

The study by Hansel et al. [11] demonstrated an association between bacterial vaginosis in the case of pregnant women and deficiency of vitamin D. That study demonstrated that the vitamin D level below 30 ng/ml increased the risk of bacterial vaginosis (BV) 2.87 times, and that was supported by some earlier reports [11]. The study by Bodnar et al. [12] indicated that vitamin D deficiency significantly reduced the risk of BV in the group of black pregnant women, but no association was confirmed in the group of white pregnant women. However, it should be remembered that BV occurred more often in the case of black women, and it was known that black women were at 6-time greater risk of vitamin D deficiency [12].

The effect of vitamin D on duration of pregnancy is explained by its influence on the immunological system. There are studies suggesting the role of vitamin D in the process of placenta implantation and in immunosuppressive processes occurring in the maternal organism [13]. Vitamin D participates in immunological processes and in response of the immunological system to bacterial invasion, which may indirectly influence duration of pregnancy [14]. Cytokines released by maternal and fetal organisms in response to bacterial invasion lead to production of prostaglandins, and then trigger a preterm contractions. Increased amniotic levels of cytokines, including interleukin-1 (Il-1), Il-6 and tumor necrosis factor α (TNF-α), were found in preterm deliveries [15]. Another factor indicating a possible effect of vitamin D deficiency on occurrence of preterm delivery is increased level of vitamin D binding protein (DBP) in vaginal secretion of women who had preterm birth. Increased DBP level during pregnancy may impair functions of vitamin D metabolites [16]. Moreover, vitamin D participates in production and influences secretion of cathelicidins, being a product of neutrophil degranulation and demonstrating bactericidal properties, as well as influences the activation of inflammation by pro-inflammatory cells [17].

Potential limitations of our report include the following. First limitation of the study is that 25(OH)D levels were obtained only from serum samples taken at the day of delivery. The levels of vitamin D throughout pregnancy were unknown, as was the impact of vitamin D status during first and second trimesters on the duration of pregnancy. Second limitation is the fact that in the studied population only few patients had serum 25(OH)D below 10 ng/ml. The vitamin D level below 10 ng/ml increased the risk of preterm birth in multivariate regression model, but the result was not statistically significant. This result was close to statistical significance, therefore the association between severe vitamin D deficiency and preterm birth needs a further study.

CONCLUSIONS

Considering a common vitamin D deficiency among pregnant women, introduction of a routine determination of serum vitamin D levels should be considered, especially in the case of pregnant women at high risk of preterm birth. Doctors should inform pregnant women that supplementation of vitamin D and proper daily exposure to sunlight are known practices which increase chances of giving birth a healthy child on time.

REFERENCES

1. Hall E, Greenberg J. Estimating community-level costs of preterm birth. Public Health. 2016;141:222–8, https://doi.org/10.1016/j.puhe.2016.09.033.
2. Bartoszewicz Z, Kondracka A, Krasnodebska-Kiljańska M, Niedźwiedzka B, Popow M, Ambroziak U, et al. Vitamin D insufficiency in healthy pregnant women living in Warsaw. Ginekol Pol. 2013;84:363–7, https://doi.org/10.17772/gp/1590.
3. Dror DK. Vitamin D status during pregnancy: Maternal, fetal, and outcomes. Curr Opin Obstet Gynecol. 2011;23:422–6, https://doi.org/10.1097/GCO.0b013e32834cb791.
4. Aydogmus S, Kelekci S, Aydogmus H, Eriş S, Desdicioğlu R, Yilmaz B, et al. High prevalence of vitamin D deficiency among pregnant women in a Turkish population and impact on perinatal outcomes. J Matern Fetal Neonatal Med. 2015;28:1828–32, https://doi.org/10.3109/14767058.2014.969235.
5. Shibata M, Suzuki A, Sekiya T, Sekiguchi S, Asano S, Udagawa Y, et al. High prevalence of hypovitaminosis D in pregnant Japanese women with threatened premature delivery. J Bone Miner Metab. 2011;29(5):615–20, https://doi.org/10.1007/s00774-011-0264-x.
6. Thorp JM, Camargo CA, McGee PL, Harper M, Klebanoff MA, Sorokin Y, et al. Vitamin D status and recurrent preterm birth: A nested case-control study in high-risk women. BJOG. 2012;119:1617–23, https://doi.org/10.1111/j.1471-0528.2012.03495.x.

7. Bodnar LM, Rouse DJ, Momirova V, Peaceman AM, Sciscione A, Spong CY, et al. Maternal 25-hydroxyvitamin D and preterm birth in twin gestations. Obstet Gynecol. 2013;122: 91–8, https://doi.org/10.1097/AOG.0b013e3182941d9a.
8. Krela-Kaźmierczak I, Szymczak A, Łykowska-Szuber L, Eder P, Stawczyk-Eder K, Klimczak K, et al. The importance of vitamin D in the pathology of bone metabolism in inflammatory bowel diseases. Arch Med Sci. 2015;11:1028–32, https://doi.org/10.5114/aoms.2015.54858.
9. Płudowski P, Kaczmarkiewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe – Recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol. 2013;64:319–27, https://doi.org/10.5603/EP.2013.0012.
10. World Health Organization [Internet]. Geneva: The Organization; 2012 [cited 2016 Sept 5]. Guideline: Vitamin D supplementation in pregnant women. Available from: http://www.who.int/publications/guidelines/en.
11. Hensel KJ, Randis TM, Gelber SE, Ratner AJ. Pregnancy-specific association of vitamin D deficiency and bacterial vaginosis. Am J Obstet Gynecol. 2011;204:41.e1–9, https://doi.org/10.1016/j.ajog.2010.08.013.
12. Bodnar LM, Krohn MA. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. J Nutr. 2009;139(6):1157–61, https://doi.org/10.3945/jn.108.103168.
13. Liu NQ, Hewison M. Vitamin D, the placenta and pregnancy. Arch Biochem Biophys. 2012;523:37–47, https://doi.org/10.1016/j.abb.2011.11.018.
14. Liu N, Kaplan AT, Low J, Nguyen L, Liu GY, Equils O, et al. Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway. Biol Reprod. 2009;80:398–406, https://doi.org/10.1095/biolreprod.108.073577.
15. Hillier SL, Witkin SS, Krohn MA, Watts DH, Kiviat NB, Eschenbach DA. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. Obstet Gynecol. 1993;81:941–8.
16. Salle BL, Delvin EE, Lapillonne A, Bishop NJ, Glorieux FH. Perinatal metabolism of vitamin D. Am J Clin Nutr. 2000;71:1317S–24S.
17. Liu N, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311:1770–3, https://doi.org/10.1126/science.1123933.