Vitamin D insufficiency in healthy pregnant women living in Warsaw

Niedobór witaminy D u zdrowych kobiet ciężarnych mieszkających w Warszawie

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Abstract

Background/Objectives: *Ensuring the optimal level of 25-hydroxy-vitamin D (25OHD) in serum (concentration above 30 ng/ml) is essential for protecting the health of the mother and the developing fetus. Vitamin D plays an important role in maintaining proper bone structure, preventing infections, reducing the risk of premature birth and gestational diabetes. The aim of the study was to verify whether healthy pregnant residents of Warsaw were deficient in vitamin D.*

Material and methods: *The material consisted of 150 serum samples of 50 healthy women in 1st, 2nd and 3rd trimester of pregnancy. 72.7% of the sera were from women who reported taking multivitamin supplements containing vitamin D_3 (71% out of that group was taking 400 IU daily). The concentration of 25OHD was measured using the vitamin D total assay on Elecsys 2010 automatic analyzer (Roche Diagnostics).*

Results: *The average serum 25OHD concentrations of 50 women in 1st, 2nd and 3rd trimester of pregnancy were respectively: 23.1 ng/ml, 24.8 ng/ml, and 25.1 ng/ml, with no statistically significant differences. The optimal levels of 25OHD (30-80 ng/ml) were found in 30.0% of samples, hypovitaminosis (20-30 ng/ml) occurred in 38.7%, deficiency (10-20 ng/ml) in 24.0% and severe deficiency (less than 10 ng/ml) in 7.3% of cases. Mean concentration of 25OHD in winter season (October 1 – March 31) was 23.6 ng/ml and in summer season (April 1 – September 30) was 25.5 ng/ml, with no statistically significant difference. On the basis of the BMI in 1st trimester two subgroups were distinguished from the studied subjects: BMI <21 (13 patients, 39 samples) and BMI >25 (14 patients, 42 samples). Mean 25OHD concentration in these groups were 27.3 and 23.5 ng/ml respectively ($p<0.05$). High statistical significance ($p<0.001$) was found among the total number of samples with 25OHD deficiency and severe deficiency (<20 ng/ml) and samples with hypovitaminosis and optimal 25OHD level (>20 ng/ml) in these groups.*

Conclusions: *Regardless of trimester and season, vitamin D below the optimal level is a common occurrence during pregnancy and the current level of supplementation among Polish pregnant women appears to be insufficient. Our data suggest that special attention should be paid to the problem of vitamin D insufficiency in overweight pregnant women.*

Key words: **vitamin D insufficiency** / **calcidiol** / **pregnancy** /

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Streszczenie

Wprowadzenie: *Zapewnienie optymalnego stężenia 25-hydroksy-witaminy D (25OHD) w surowicy kobiet ciężarnych jest konieczne dla ochrony zdrowia matki i dziecka. Witamina D bierze udział w tworzeniu kości, przeciwdziała infekcjom, zmniejsza ryzyko przedwczesnych porodów i cukrzycy ciążowej.*

Celem pracy było sprawdzenie czy u zdrowych kobiet ciężarnych mieszkających w Warszawie występuje niedobór witaminy D.

Materiał i metody: *Materiał stanowiło 150 surowic od 50 zdrowych kobiet w I, II i III trymestrze ciąży. 72,7% surowic pochodziło od kobiet przyjmujących preparaty wielowitaminowe zawierające witaminę D_3 (71% z nich przyjmowało 400 IU witaminy D dziennie). Stężenie 25OHD mierzono testem vitamin D total na automatycznym analizatorze Elecsys 2010 (Roche Diagnostics).*

Wyniki: *Średnie stężenie 25OHD w surowicy kobiet w I, II i III trymestrze ciąży wyniosło odpowiednio: 23,1 ng/ml, 24,8 ng/ml i 25,1 ng/ml. Optymalny poziom 25OHD zaobserwowano w 30,0% próbek, hipowitaminozę w 38,7%, deficyt w 24,0% oraz głęboki deficyt w 7,3% próbek. Średnie stężenie 25OHD w sezonie zimowym nie wykazywało statystycznie znamiennych różnic w porównaniu z sezonem letnim i wyniosło odpowiednio 23,6 ng/ml i 25,5 ng/ml. U kobiet z BMI w I trymestrze ciąży <21 kg/m^2 (13 pacjentek, 39 próbek) i BMI >25 kg/m^2 (14 pacjentek, 42 próbek) średnie stężenie 25OHD wyniosło odpowiednio 27,3 ng/ml i 23,5 ng/ml. W tych grupach zaobserwowano wysoką znamienność statystyczną (p<0.001) pomiędzy całkowitą liczbą próbek z deficytem oraz głębokim deficytem 25OHD (<20 ng/ml) w porównaniu z liczbą próbek z hipowitaminozą i optymalnym poziomem 25OHD (>20 ng/ml).*

Wnioski: *Niezależnie od trymestru oraz pory roku stężenia witaminy D poniżej poziomu optymalnego jest częste wśród kobiet ciężarnych w Polsce a obecnie stosowany poziom suplementacji wydaje się niewystarczający. Nasze wyniki sugerują, że większą uwagę należy zwrócić na problem niedoboru witaminy D wśród kobiet ciężarnych z nadwagą.*

Słowa kluczowe: **niedobór witaminy D** / **kalcydiol** / **ciąża** /

Introduction

Ensuring an optimal level of 25-hydroxy-vitamin D (25OHD) in serum (concentration above 30 ng/ml) and adequate supplementation of vitamin D, required in case of deficiency, is especially important during pregnancy due to the role vitamin D plays in protecting the health of the mother and the developing fetus [1, 2]. High demand for calcium increases its absorption in the digestive tract which requires an additional supply of vitamin D for proper bone mineralization of the fetus, as well as synthesis of calcium binding proteins necessary for vitamin D absorption. In severe vitamin D deficiency the fetus may develop the rickets *in utero* with manifestation at birth [3]. There is also a direct link between the increased level of 25OHD in serum and the modulation of immune system in pregnancy, what may result in reducing the risk of several types of diseases related to bacterial and viral infections through the mechanism of induction of antimicrobial peptides and proteins (AMP) [4]. These include bacterial vaginosis, mother-to-child transmission (MCTC), human immunodeficiency virus (HIV), dental caries, pneumonia, severe sepsis, and tuberculosis [2, 5, 6]. Vitamin D deficiency has been associated with various complications for the mother and the fetus, including preeclampsia, gestational diabetes and premature birth [7, 8, 9]. Vitamin D supply varies in different countries depending on diet and geographic location. The Polish Gynecological Society recommends vitamin D supplementation in pregnant women [10].

It is widely accepted that vitamin D status in humans is best reflected by the measurement of serum concentration of its 25-hydroxylated form (25OHD; calcidiol). Its level accurately shows mean vitamin D supply because of long half-life of 25OHD (two-three weeks) and high, easily measured concentration. Despite the fact that the active form of vitamin D ($1,25(OH)_2D$, calcitriol) can also be measured, its short half-life, significant dependence on renal failure and low concentration in plasma (more than 1000 times lower in comparison with 25OHD) bring on technical challenge and limited usefulness of its measurement.

The aim of the study was to assess whether randomly selected healthy pregnant residents of Warsaw were deficient in vitamin D and to measure the extent of that deficit.

Material and methods

Study group

The material consisted of serum samples from 50 healthy volunteers in 1st, 2nd and 3rd trimesters of pregnancy. All women were non-smokers, had no autoimmune thyroid diseases (negative ATPO and ATG) and goiter (thyroid volume below 18 ml). Other autoimmune diseases, diabetes and gestational diabetes were excluded on the basis of medical history. All women delivered healthy children (average Apgar score 9.91, average newborn weight 3472 g) at term (average term of pregnancy 39.6 weeks). 72.7 % of the sera were obtained from women who reported taking multivitamin supplements containing vitamin D_3. In that group, 71% of women reported taking Feminatal (400 IU of vitamin D_3), 13.3% - Prenatal (800 IU), 2.4% - Elewit (500 IU), 0.8% - Prevital (250 IU) and 6.6% - Centrum or Falvit (200 IU). None of the women declared supplementation with vitamin D drops. Characteristics of the study group are presented in Table I. The study protocol was approved by the Ethical Committee of the Warsaw Medical University and written consent was obtained from all patients.

Table I. Characteristics of the study group (n=50).

Parameter	mean±sd (min-max)
Weight (kg])	
1st trimester	64.9±8.3 (49.0-87.0)
2nd trimester	68.9±8.7 (50.5-89.0)
3rd trimester	73.9±9.6 (53.0-93.0)
BMI (kg/m^2)	
1st trimester	23.4±3.1 (18.4-32.0)
Age (1st trimester), years	29.4±3.1 (24-37)
	Percentages (%)
Vit.D supplementation	
1st trimester	80%
2nd trimester	70%
3rd trimester	68%
First pregnancy	52%
Second pregnancy	36%

Biochemical measurements

Fasting blood samples were collected between 7:30 and 9:30 am. Samples for biochemical determinations were routinely centrifuged and serum was kept frozen at –20ºC until quantitative assay. The concentration of 25OHD was measured using the vitamin D total assay on Elecsys 2010 automatic analyzer (Roche Diagnostics).

Vitamin D status

According to Polish terminology, vitamin D supply in adults, based on serum 25OHD concentration, can be divided into the following categories: severe deficiency (<10 ng/ml), medium deficiency (10-20 ng/ml), hypovitaminosis D (20-30 ng/ml), recommended or optimum level (30-80 ng/ml) and toxic level (>100 ng/ml) [11, 12]. These categories differ slightly from the World Health Organization and American and Canadian classification, where levels below 10 ng/ml (USA) or below 20 ng/ml (Canada) were considered deficient and levels below 30 ng/ml were classified as insufficient. An optimal level is typically defined as serum 25OHD level between 30-76 ng/ml [13-15].

Statistical analysis

Statistical analysis was performed by Statistica version 10 (StatSoft). Parametric t-Student's and non-parametric U Mann-Whitney tests were used to compare the quantitative variables between two independent groups. V-square test was used for distribution analysis. $p<0.05$ was considered statistically significant.

Results

25OHD concentration in 150 serum samples from 50 healthy pregnant women were measured (Table II). The average concentrations of serum 25OHD in 1st, 2nd and 3rd trimester were respectively: 23.1 ng/ml, 24.8 ng/ml, and 25.1 ng/ml, with no statistically significant differences between the trimesters. Optimal levels of 25OHD (30-80 ng/ml) were found in 30.0% of the samples and the rest was below optimum level. Hypovitaminosis (20-30 ng/ml) was observed in 38.7% of the samples, medium deficiency (10-20 ng/ml) in 24.0% and severe deficiency (less than 10 ng/ml) in 7.3% of cases. 28 of 50 women declared taking multivitamin supplements containing vitamin D_3 during all three trimesters of pregnancy, four women did not use vitamins in pregnancy and nine reported supplementation only during one of the trimesters. Average concentrations of 25OHD in samples taken from women declaring supplementation with multivitamin containing vitamin D_3 in the 1st, 2nd and 3rd trimester were 23.4, 25.1 and 26.6 ng/ml, respectively, and in samples from women using no multivitamin pills 22.0, 24.1 and 22.1 ng/ml, respectively, with no statistically significant difference. Mean concentration of 25OHD during winter season (October 1 – March 31) was 23.6 ng/ml and in summer season (April 1 – September 30) it was 25.5 ng/ml, with no statistically significant difference (Table III).

Two subgroups were selected from the studied subjects according to BMI in the 1st trimester: with the lowest BMI (<21; 13 patients, 39 samples) and with the highest BMI (>25; 14 patients, 42 samples) (Table IV).

The discrepancy in body mass between both groups lasted to the end of the pregnancy – the weight gains between the first visit and 2nd and 3rd trimesters observed in low-BMI and high-BMI groups were higher in the high-BMI group (mean 4.5 and 10.3 kg versus 3.5 and 7.9 kg in low-BMI group). Mean 25OHD concentrations in these groups were significantly different (27.3 and 23.5 ng/ml respectively; $p<0.05$). Much higher percentage of optimal 25OHD results (35.9%) was found for low-BMI than for high-BMI women (23.8%). Severe deficiency was observed in none of the 39 samples coming from the low-BMI women and five of the 42 samples (11.9%) from the high-BMI group. High statistical significance ($p<0.001$) was found among the total number of samples with 25OHD deficiency and severe deficiency (<20 ng/ml) and samples with hypovitaminosis and optimal 25OHD level (>20 ng/ml) in these group.

Discussion

Although the optimal serum level of 25OHD for adults is above 30 ng/ml, according to international and Polish recommendations, population studies indicate that vitamin D insufficiency is a global epidemic [16, 17]. The prevalence of vitamin D deficiency in the Polish population remains largely unknown. Our results, coming from a group of healthy pregnant volunteers living in Warsaw, indicate that vitamin D insufficiency affected 70% of the studied individuals and average 25OHD serum concentration was 24.4 ng/ml (Table II).

During pregnancy vitamin D demand is even higher than for non-pregnant women, due to growing needs of the mother and the child [1, 3]. Thus, it is unsurprising that vitamin D supplementation is highly necessary and vitamin supplements containing vitamin D_3 or D_2 are most recommended to pregnant women. 92% of our patients declared taking vitamin supplements during pregnancy (about 400 IU D_3 daily), but only half of them did it regularly throughout the entire pregnancy and about one fourth of the women did not use vitamin supplementation or used it only incidentally. Our results clearly indicate that, regardless of gestation length and season, the level of supplementation has a minor effect on vitamin D status which is still below the optimum level (Table II and III).

Table II. Serum concentration of 25OHD and the percentage of results with optimal (>30 ng/ml) and insufficient levels of 25OHD in pregnant women taking multivitamin supplements containing vitamin D_3 (vitD sup) and pregnant women without supplementation (vitD unsup).

Parameter	1st trimester			2nd trimester			3rd trimester			All trimesters		
Group (n)	vitD sup (40)	vitD unsup (10)	all (50)	vitD sup (35)	vitD unsup (15)	all (50)	vitD sup (34)	vitD unsup (16)	all (50)	vitD sup (122)	vitD unsup (43)	all (165)
25OHD [ng/ml]												
mean	23.4	22.0	23.1	25.1	24.1	24.8	26.6	22.1	25.1	24.9	22.8	24.4
sd	9.4	5.9	8.9	9.2	9.6	9.3	10.1	10.2	10.4	9.7	9.1	9.6
min	7.9	7.4	7.4	8.1	6.1	6.1	7.8	3.3	3.3	7.6	3.3	3.3
max	47.3	31.9	47.3	44.2	40.9	44.2	49.7	37.4	49.7	49.7	40.9	49.7
25OHD [%]												
>30 ng/ml	25.0	10.0	22.0	31.4	33.3	32.0	38.2	31.3	36.0	31.2	26.8	30.0
20-30 ng/ml	37.5	70.0	44.0	31.7	40.0	38.0	35.3	31.3	34.0	36.7	43.9	38.7
10-20 ng/ml	30.0	10.0	26.0	28.6	13.3	24.0	23.5	18.8	22.0	27.5	14.6	24.0
<10 ng/ml	7.5	10.0	8.0	2.9	13.3	6.0	2.9	18.8	8.0	4.6	14.6	7.3

Table III. Serum 25OHD concentration and the percentage of results with optimal (>30 ng/ml) and insufficient levels of 25OHD in the study group in the winter (October 1 – – March 31) and summer season (April 1 – September 30) in pregnant women taking multivitamin supplements containing vitamin D_3 (vitD sup) and pregnant women without supplementation (vitD unsup). In brackets, the number of patients.

Parameter	Summer			Winter		
	vitD sup (42)	vitD unsup (15)	all (57)	vitD sup (67)	vitD unsup (26)	all (93)
25OHD [ng/ml]						
mean	26.6	22.5	25.5	23.9	22.9	23.6
sd	9.6	9.2	9.7	9.5	9.1	9.4
min	7.9	3.3	3.3	7.6	6.1	6.1
max	47.3	34.7	47.3	49.7	40.9	49.7
25OHD [%]						
>30 ng/ml	38.1	33.3	36.8	26,9	23.1	25.8
20-30 ng/ml	35.7	40.0	36.8	37.3	46.2	39.8
10-20 ng/ml	23.8	13.3	21.1	29.9	15.4	25.8
<10 ng/ml	2.4	13.3	5.3	6.0	15.4	8.6

Current Polish recommendations for vitamin D supplementation during pregnancy are 800–1000 IU/day, but such doses are rarely taken by patients. The weakness of these recommendations lies in the fact that they are based almost exclusively on the results of authors studying populations other than Polish [18, 19].

Moreover, multivitamin pills for pregnant women in Poland usually contain 400 IU of vitamin D_3 or less. In our study, 71% of women reporting taking 400 IU/day, only 13.3% 800 IU/day and none of the women declared doses above 800 IU/day. The suggestion of Polish experts that the optimal procedure during pregnancy and breastfeeding is to adjust individual doses of vitamin D to each patient [18] is not implemented in routine practice. The American Committee of Obstetric Practice and Gynecologists recommends vitamin D doses of 1000-2000 IU daily if vitamin D deficiency is diagnosed during pregnancy [14]. Recent studies of Hollis et al., report that administration of 2000-4000 IU of vitamin D per day during the second and third trimesters of pregnancy causes substantial increase in serum 25OHD concentration without hypercalcemia, whereas supplementation with 400 ID/day has only a minor effect [1, 20]. Thus, these authors concluded that current international recommendations for vitamin D supplementation during pregnancy should be raised to 4000 IU/day [20]. Nevertheless, due to the absence of evidence for differences in clinical outcomes regarding vitamin D dose or serum 25OHD levels, other researchers are more inclined to postulate 'more controlled trials with biologically meaningful endpoints determined' before the recommendations are revised [21].

The question remains how to supplement vitamin D. Multivitamin supplements are probably not the best source of vitamin D because the absorption of hydrophobic vitamin D may be significantly lower than the other vitamins and elements, well-soluble in an aqueous medium [22]. Also, stability of vitamin D in multivitamin pills is unknown. Potentially better absorbed preparations containing exclusively vitamin D are not widely used by patients in Poland. Therefore, it is necessary to change the awareness of pregnant women by providing more information about the importance of ensuring optimal levels of vitamin D for the health of the mother and the child.

Table IV. Percentage of pregnant women with optimal (>30 ng/ml) and insufficient 25OHD serum concentration (1st, 2nd and 3rd trimester results) in groups with low BMI (<21) and high BMI (>25). BMI was calculated in the 1st trimester; the number of women and serum samples are shown.

25OHD level	Low BMI n=13/39	High BMI n=14/42
25OHD [ng/ml]		
mean	27.3*	23.5*
sd	7.6	10.4
min	12.9	3.3
max	47.3	49.7
25OHD [%]		
>30 ng/ml	35.9	23.8
20-30 ng/ml	48.7	40.5
10-20 ng/ml	15.4	23.8
<10 ng/ml	nd	11.9

nd – not detected
*p<0.05 (non-parametric U Mann-Whitney test)

Surprisingly, our results revealed minor seasonal changes of 25OHD concentrations (25.5 ng/ml in summer versus 23.6 ng/ml in winter) that are difficult to explain and contrary to the reports in the literature [23]. One of the possible explanations could be the fact that pregnant women avoid high sun exposure in the summer, between 10 am and 2 pm, when the sun is most effective in vitamin D skin synthesis at this latitude, because of their economic activity, often lasting to the end of pregnancy. Moreover, little influence of declared supplementation on mean 25OHD concentration was noted (Table III). Extremely low 25OHD was observed in both groups of pregnant women declaring and denying supplementation, what further supports the conclusion that supplementation at 400 IU/ daily is not sufficient enough to significantly rise the concentration of 25OHD in serum [20].

The dependence between obesity and vitamin D supply was analyzed on the basis of BMI, calculated according to the weight and height values obtained during the first visit of a pregnant woman (in the 1st trimester), what is common practice in population studies of pregnant women [24]. The first visit's BMI is usually used for practical reasons as the pre-pregnancy weight declared by women is often not verifiable [25]. Vitamin D level in the studied subjects, who were assigned in the 1st trimester of pregnancy to either low- or high-BMI subgroups, revealed significant inverse relationship between serum 25OHD concentration and BMI (Table IV). That effect can be due to the mechanism of "sequestration" of vitamin D in the excess of adipose tissue. Our results support the data that obesity may lead to poor vitamin D status during pregnancy [26,27] and focus on the problem of vitamin D insufficiency in overweight pregnant women. It seems that more detailed studies on controlling vitamin D status and its supplementation in a large group of Polish pregnant women should be conducted.

Conclusions

Regardless of trimester and season, vitamin D below the optimal level is a common finding in pregnant residents of Warsaw. The current level of supplementation during pregnancy through multivitamin tablets (typically containing 400 IU vitamin D_3) in Poland appears to be insufficient.

Our data suggest that special attention should be paid to the issue of vitamin D insufficiency in overweight pregnant women.

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