

Vitamin D Level in Pregnant Women Depends on Age and Weight Before and During Pregnancy

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Abstract: The AIM of our study was to evaluate the association between vitamin D levels and age, body weight before pregnancy and during the screening and the term of pregnancy. **MATERIALS AND METHODS:** We investigated 547 unselected pregnant Bulgarian women, mean age 30 ± 5 years, median 30 years (18-47 years). The distribution of pregnant women according to their age is as follows: 18-22 y – 33 (6%), 23-27 y – 115 (21%), 28-33 y – 219 (40%), 33-37 y – 128 (23.4%), 38-42 y – 46 (8%), 43-47 y – 6 (1.1%). The allocation according to trimesters of pregnancy was: first trimester - 111 (20.3%), second trimester - 275 (50.4%), and third trimester - 161 (29.3%). In the current study we introduced four categories of 25 (OH) D - <10, 10-20, 20-30 and >30 ng/mL. We evaluated the body weight prior to conception and during pregnancy. The peripheral levels of 25 (OH) D were investigated using a standard assay in a central laboratory on the day of the sampling. **RESULTS:** We found significant correlation between vitamin D levels and age ($P < 0.0001$), negative correlation with the BMI prior to conception ($P < 0.001$) and at the time of the screening ($P < 0.0001$), but no significant differences among the three trimesters concerning vitamin D levels. **CONCLUSIONS:** The levels of vitamin D among pregnant Bulgarian women are within the span of mild insufficiency, probably due to the normal BMI before and during the pregnancy. We noticed that with the increase of age, the more mature pregnant women have more responsible behavior and follow the advice of their obstetrician/gynecologist.

Keywords: Vitamin D, Pregnancy, Gestational Age, Body Mass Index, Age

1. Introduction

In the winter of 2012 Bulgarian Society of Endocrinology undertook a cross-sectional nested study on the levels of vitamin D and parathyroid hormone (PTH) among 2033 Bulgarians in 12 cities and the adjacent villages [1]. This study defined the threshold value of 25-hydroxyvitamin D [25 (OH) D] below which PTH levels increase - 50 nmol/L (20 ng/mL), i.e. this is the level of 25 (OH) D needed to suppress PTH secretion. In this study 24.2% of the population had 25 (OH) D levels above 50 nmol/L (20 ng/mL) in the winter. In Bulgaria the food is not boosted with vitamin D and the majority of the adult population in this study have

moderate to severe vitamin D deficiency. Moreover, this deficiency is more pronounced among females with no significant differences among different age groups. During the same year an additional seasonal follow-up of vitamin D levels was undertaken. This study encompassed part of the previously investigated individuals who had vitamin D deficiency or insufficiency. The investigation revealed that 8.5% of the studied population had vitamin D deficiency during the summer [2]. The levels of vitamin D were investigated in nursing homes for elderly and were compared to those of elderly individuals living in their own homes [3]. The comparative analysis showed significant differences between the two groups. Still, there is no data concerning the levels of vitamin D in pregnant women. These levels

represent an important social issue.

According to the literature, the prevalence of vitamin D deficiency differs and depends on many factors, such as the season of the investigation, geographic characteristics, race, skin colour, food and living habits, etc. In a 2014 study of the Royal College of Obstetricians & Gynaecologists, 47% of the Asian, 64% of the Middle East, 58% of the black women and 13% of the Caucasian women living in London had vitamin D levels below 25 nmol/L [4]. Therefore, every screening program for vitamin D deficiency should be organized in accordance with the specific characteristics of the investigated population and the results retrieved are valid only for this particular population.

The aim of current study was to define the mean levels of 25 (OH) D in pregnant Bulgarian women, to evaluate the prevalence of moderate to severe deficiency and the associations between these levels and the age, body weight before pregnancy and during the screening and with the term of pregnancy.

2. Study Design

We undertook a multicenter cross-sectional population-based study performed between September 25 and November 6 2019 in 10 Bulgarian cities (41°–44°N) with the adjacent villages (overall 84 settlements): Sofia, Samokov, Pirdop, Smolian, Gotze Delchev, Gabrovo, Troian, Burgas, Stara Zagora, Pleven. We established communication with 104 specialists in Endocrinology and Obstetrics and Gynecology, who invited more than 630 pregnant women to participate in the study. Overall 547 pregnant women consented (86.83%).

3. Materials and Methods

We investigated 547 pregnant women, mean age 30 ± 5 years, median 30 years (18-47 years). The distribution of pregnant women according to their age is as follows: 18-22 y – 33 (6%), 23-27 y – 115 (21%), 28-33 y – 219 (40%), 34-37 y – 128 (23.4%), 38-42 y – 46 (8%), 43-47 y – 6 (1.1%). The allocation according to trimesters of pregnancy was: first trimester - 111 (20.3%), second trimester - 275 (50.4%), and third trimester - 161 (29.3%). Prior to the study, all participants signed an informed consent approved by the local Ethics committee at the University Hospital Sofamed (Protocol 14/16 Sept 2019), and the research was conducted in accordance with the Declaration of Helsinki. The investigated pregnant women were a random cohort, without pre-selection. All pregnant women filled in a questionnaire. Overall 457/547 (83.55%) were taking medication (s) at the time of the investigation, mainly vitamin supplements 278/547 (50.82%) as a monotherapy or in combination with other medications. All participants were Caucasians and none had liver or kidney diseases or malabsorption. The study did not require a sun exposure diary. During the previous months all pregnant women adhered to their usual daily routine.

In a previous population-based study [1], using adaptive

regression analysis on the effect of 25 (OH) D on PTH levels, we defined a cut-off value for hypovitaminosis D of 20 ng/mL (50 nmol/L). In this project was to determine both vitamin D deficiency in the Bulgarian population as a whole and its connection with gender and age. The levels below 10 ng/mL (25 nmol/L) were referred to as severe deficit, 10 - 20 ng/mL (25 - 50 nmol/L) – a deficit similar to the criteria adopted at the Conference on this topic in Eibsee, Germany in 2012 [5]. In our current study we accepted sufficiency level above 30 ng/mL (75 nmol/L) and introduces additional degree of vitamin D insufficiency – between 20 and 30 ng/mL (50-75 nmol/L), based on the IOM recommendations [6] and Endocrine Society clinical practice guideline [7]. Whereas, the IOM recommendations define sufficiency as vitamin levels above 50 nmol/L (20 ng/mL), the second guidelines suggest levels above 75 nmol/L (30 ng/mL), and both recommendations are in agreement with other expert opinions in the field [8, 9], that the optimum levels of vitamin D should be 75 – 125 nmol/L (≥ 30 ng/mL) in order to preserve bone health. The latter is crucial during pregnancy for the formation of the fetal skeleton. Therefore, in pregnant women we suggest the following categories of 25 (OH) D levels:

1. Severe deficiency (deficit): < 10 ng/mL
2. Moderate deficiency (deficit): 10 - 20 ng/mL
3. Insufficiency: 20 - 30 ng/mL
4. Sufficiency: > 30 ng/mL

All studied pregnant women filled in a specially designed questionnaire, concerning the course of the current pregnancy and pre-conception history (a structured questionnaire including demographic data, personal and family medical history, thyroid disorders, diabetes and chronic renal disease, past and current medication, vitamin D or other vitamin intake, etc.).

In every investigated pregnant woman we measured the body weight and height and the body weight before conception was documented in the Questionnaire. Based on the respective documented values, the body mass index (BMI) before conception and at the time of the investigation was calculated.

3.1. Laboratory Test

Morning blood sample was taken from the cubital vein and the total levels of 25 (OH) D were determined using standard Electro Chemoluminescence Immuno Assay (Competition ECLIA method on Cobas 601 analyzer) in a central laboratory on the day of the sampling. Serum tubes containing separating gel were used. The samples were transported to the lab after centrifugation. The results are determined via a calibration curve in ng/mL. Two levels Intralaboratory quality control on a daily basis was performed. The Laboratory is participating in two EQA systems – Bulgarian EQAS and INSTAND and has certificates for this parameter.

3.2. Statistical Processing

The data were analyzed with SPSS for Windows v.13.0. descriptive statistics (mean, median values, standard

deviation), correlation and dispersion analysis (using parametrical and non-parametrical methods, including Student's t-test, Kruskal-Wallis test and Mann-Whitney test.), ANOVA testing, post-hoc test - Bonferroni. All values were presented as mean values with standard deviations, median or percentage (unless specified otherwise), p values below 0.05 were accepted as statistically significant.

4. Results

Overall 84% of the investigated pregnant Bulgarian women were aged 23 - 37 years, half of them being 28 - 32 years of age. A lower percentage of women were between 38 and 42 years (8%) or 18 - 22 years (6%), and a very small percentage were in the age group 43 - 47 years (1.1%).

For the whole group (547 pregnant women), the mean 25 (OH) D level was 25.86 ± 9.46 ng/mL; median 24.51 (7.96 - 70.00), corresponding to mild insufficiency during the autumn. Table 1 presents the four groups according to vitamin D levels – severe deficiency, deficiency, insufficiency and sufficiency.

Table 1. The prevalence of different four level vitamin D – severe deficiency, deficiency, insufficiency, sufficiency in cohort of pregnant women.

Category (group)	25 (OH) D level (ng/ml)	Number	%
1 Severe deficiency	<10	7	1.28
2 Deficiency	10-20	141	25.78
3 Insufficiency	20-30	251	45.88
4 Sufficiency	≥ 30	148	27.06

Only 27.06% of the investigated pregnant women had sufficient levels of 25 (OH) D above 30 ng/L (group 4) and the other three groups (1, 2 and 3) had deficiency or insufficiency-72.94%. The mean age of the investigated cohort was 30.49 ± 5.12 years. The group 4 (n=148) with 25 (OH) D ≥ 30 ng/mL was aged 31.39 ± 5.02 years, compared to 30.16 ± 5.13 years for groups 1 plus 2 plus 3 (n = 399), where 25 (OH) D levels were below 30 ng/mL ($P < 0.02$).

The mean BMI before pregnancy for the whole cohort was 23.57 ± 6.9 kg/m²; median 22.15 (15.24-133.33), at the screening (i.e. during pregnancy) - 26.15 ± 7.28 kg/m²; median 25.30 (16.51-147.22).

The mean body weight before pregnancy for the whole cohort was 63.68 ± 14.36 kg, at the screening (during pregnancy) - 70.60 ± 14.50 kg. Before the pregnancy the mean weight of group 4 was 61.27 ± 11.89 kg, and for the other three groups - 64.57 ± 15.09 kg ($P < 0.034$). The respective body weight at the screening was 67.45 ± 12.79 kg in group 4 compared with 71.76 ± 14.94 kg for groups 1 plus 2 plus 3 ($P < 0.002$).

The average levels of vitamin D in the first, second and third trimesters were 25.97 ± 10.28 , 25.34 ± 8.89 and 26.70 ± 9.84 ng/mL, respectively, with no significant differences between the subgroups (NS).

We found significant correlation of vitamin D levels and: age [Spearman's rho = 0.151, $P < 0.0001$], negative correlation with BMI before pregnancy [Spearman's rho = -

0.142, $P < 0.001$] and BMI at the screening [Spearman's rho = - 0.171, $P < 0.0001$]; and no correlation between vitamin D levels and the gestational age ($P = \text{NS}$).

5. Discussion

In their study on the role of vitamin D supplementation during pregnancy, Luz Maria De-Regil *et al.* (2019) suggest that during pregnancy decreases the risk of preeclampsia, gestational diabetes and low fetal birth weight [10]. This is in harmony with the findings of other studies on vitamin D in pregnancy [11, 12]. Despite the unclear role of vitamin D supplementation, all authors are in accordance that such prevention should be administered [4].

In our pregnant cohort, the first thing that could be noted is the change in the age profile of the Bulgarian pregnant women in the past few years. Compared to the 2015 Euro-Peristat Project population [13], five years later, in 2019, the percentage of pregnancies below the age of 20 has decreased in Bulgaria (from 10.2% to 2.74%, respectively), as well as in the range between 20 and 24 years (from 21.7% to 7.9%, respectively). At the same time there is an increase in the percentage of pregnant women between 25 and 34 years (from 54.5% to 68.52%) and 35+ years (from 13.6% to 20.84%, respectively), figure 1.

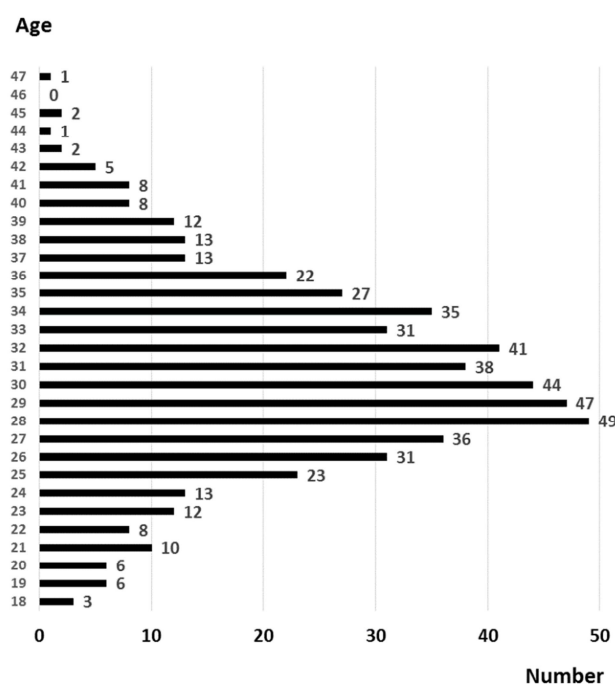


Figure 1. Age distribution of 547 pregnant Bulgarian women - 2019.

What is the importance of this phenomenon? The described shift in the age distribution is a good sign for maturation of the society as a whole. The well informed and educated adult female individual with experience could take better care for the desired pregnancy. A confirmation of this hypothesis is the fact that in group 4 (with the highest levels of 25 (OH) D ≥ 30 ng/mL) the mean age is significantly

higher than in groups 1, 2 and 3 (who have vitamin D insufficiency): 31.39 ± 5.02 years vs 30.16 ± 5.13 years, respectively, $P < 0.02$. Moreover, the “older” young women desire pregnancy more and have more interest in taking adequate pregnancy care. These more mature women better understand their higher responsibility towards the unborn baby and change their lifestyle and habits, undertake investigations, consultations and take medications and supplements if needed. These women have better values of two other indicators in the pregnancy questionnaire – smoking during the current pregnancy and normal BMI pre-conception, both included in the Euro-Peristat Project, 2015 [13]. Both indicators have been thoroughly discussed by J. Zeitlin et al [14].

Concerning the mean 25 (OH) D levels of 25.86 ± 9.46 ng/mL (median 24.51, 7.96 - 70.00), we could conclude that there is a mild insufficiency within the whole group of 547 pregnant women. We cannot compare the population results from September-October 2012 (52.75 nmol/L = 21.18 ng/mL) with the results of our present study, because of the different demographics in both investigations: the 2012 study included men and women aged 20-80 years, the selection of the participants was different (individuals with pre-diagnosed deficiency / insufficiency in winter, who were instructed **not** to take vitamin D supplements). In general, the levels of 25 (OH) D are lower in women, but higher in younger age. Moreover, 30% of the studied pregnant women are from small towns and villages where the mean levels of vitamin D are higher [15].

Another study of pregnant women in Belgium (46°N) was performed between September and June and vitamin D levels were followed up during different trimesters, but some of the pregnant women were of North-African descend. Again, because of the different design and the presence of two races, different lifestyles, food habits and body coverage of the investigated women the results are not comparable with our study. Moreover, Belgium is located more to the North, and 74% of the women have vitamin D insufficiency [25 (OH) D < 30 ng/mL], 44% have deficiency [25 (OH) D < 20 ng/mL], and 12% have severe deficiency [25 (OH) D < 10 ng/mL] [16]. These examples only illustrate the two phenomena, observed in our study: 1) Severe deficiency is observed in a different percentage of the pregnant women (12% in Belgium versus 1.28% in our study); 2) All studies (including our investigation) have used the same criteria for the evaluation of vitamin D status with the universal cut-off points recommended by the relevant international authorities.

The negative correlation between the body weight and the levels of vitamin D is a well-known fact. Probably this is one of the factors determining the higher 25 (OH) D levels in group 4 where the body weight was lower both before and during pregnancy compared to that in groups 1, 2 and 3 (before pregnancy - 61.27 ± 11.89 kg vs 64.57 ± 15.09 kg, $P < 0.034$; during pregnancy - 67.45 ± 12.79 kg vs. 71.76 ± 14.94 kg, $P < 0.002$). An additional factor is the weight gain during pregnancy – 6 kg in group 4 and 7 kg in groups 1, 2 and 3, respectively. We should underline that both before and

during pregnancy the investigated women had normal mean BMI (23.57 ± 6.9 and 26.15 ± 7.28 kg/m², respectively).

In our study, the role of age, body weight and the intake of vitamin supplements on vitamin D levels during pregnancy is clear, and the term of pregnancy does not affect vitamin D status.

6. Conclusion

The levels of vitamin D among pregnant Bulgarian women are within the span of mild insufficiency, probably due to the intake of combined vitamin supplements by approximately half of the studied women, and the normal BMI before and during the pregnancy. The more mature age at the time of pregnancy and the more responsible behavior and care aid the adherence to the advice given by their obstetrician/gynecologist.

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Conflicts of Interests

All the authors do not have any possible conflicts of interest.

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