

RECENZION
from
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on the dissertation work
for obtaining the educational and scientific degree "Doctor"
on
Dr. Monika Toshkova Todorova
ROLE OF VITAMIN D AND VITAMIN B12 IN PREGNANT WOMEN AND
NEWBORNS
for
awarding of an educational and scientific degree "DOCTOR"
Supervisor: **Assoc. Prof. Daniela Ivanova Gerova, MD, PhD**
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It is known that during pregnancy, the health status and lifestyle of the pregnant woman are essential for the normal development of the fetus and the birth of a healthy newborn.

There is a lot of evidences that vitamin D deficiency is common among pregnant women worldwide. Vitamin D deficiency is associated with pregnancy complications such as gestational diabetes mellitus, preeclampsia, premature birth. The deficiency also affects newborns, with low birth weight, neonatal death and stillbirth being common abnormalities. In Bulgaria, there is very limited data regarding the vitamin D status of pregnant women and the frequency of vitamin D deficiency/insufficiency among this target group. There is a lack of data on the relationship between vitamin D deficiency and possible complications occurring during pregnancy.

Increasingly, vitamin B12 deficiency is also found among pregnant women. The most common maternal complications are gestational diabetes, preeclampsia, miscarriages and depression. Signs of B12 deficiency on the part of the newborn are associated with neural tube defects, fetal growth disorders, retardation in neuropsychological development. The actual determination of vitamin B12 status depends not only on total vitamin B12, but also on several additional parameters such as active B12 and the metabolic indicators methylmalonic acid and homocysteine. In Bulgaria, there is a lack of data both on the vitamin B12 status of pregnant women and on the relationship between vitamin B12 deficiency and complications occurring during pregnancy.

There are relatively few studies on the analytical reliability of the methods examining these indicators. The accumulation of new information on these problems will facilitate the interpretation of the obtained results and will support the diagnosis, monitoring and follow-up of pregnant women. This defines the topic of the work proposed for review as current, contemporary and significant.

The dissertation is written on 187 standard pages. It is structured as follows: title page, list of used abbreviations and content text of the dissertation, grouped into chapters: introduction – 3 pages, literature review – 51 pages, aim and tasks – 2 pages, material and method – 10 pages, results – 46 pages, discussion – 32 pages, conclusions – 2 pages, contributions – 2 pages. The dissertation contains 34 figures and 59 tables. 350 literary sources are cited, of which 5 in Cyrillic, 342 in Latin and 3 internet sites. Most of the articles published in the last ten years.

The **literature review** includes four sections, ending with a summary. First of all, pregnancy is examined as a special physiological state. Attention is paid to pathological pregnancy and adverse complications for the mother and the newborn. The pathophysiological mechanisms of the occurrence and development of gestational diabetes mellitus, preeclampsia, premature birth, birth of children with low weight, intrauterine retardation of the fetus are monitored. In the next section, the biosynthesis and metabolism of vitamin D is discussed. The analytical reliability of immunological and chromatographic methods for determining vitamin D status is compared. Limits for deficiency, insufficiency, and normal values for vitamin D are presented. Recommendations for supplementation with the vitamin are highlighted. Attention is paid to vitamin D metabolism in healthy pregnant women. A report on the incidence of vitamin D deficiency in pregnant women is presented. The effects of vitamin D deficiency on the course of pregnancy and fetal development are discussed. The relationship between vitamin D deficiency and a number of deviations - insulin resistance, gestational diabetes, preeclampsia, bacterial vaginosis, postpartum depression, premature birth, low birth weight - was examined.

The other micronutrient under review is Vitamin B12. The biosynthesis, metabolism, and physiological role of vitamin B12 are reviewed, as well as factors determining vitamin B12 requirements. The importance of the vitamin in the processes of hematopoiesis and the development of the central and peripheral nervous system is emphasized. The role of Vitamin B12 in the occurrence of socially significant diseases such as heart attacks, strokes, malignant tumors is highlighted. The incidence of vitamin B12 deficiency in the general population is tracked and information on the recommended daily intake of the vitamin is presented. Analytical methods for determining vitamin levels are reviewed.

The review examines in detail the effects of vitamin B12 deficiency on the course of pregnancy and fetal development. The association between vitamin B12 deficiency and gestational diabetes, preeclampsia, fetal malformations, preterm birth and low birth weight has been reviewed.

In general, the literature review shows good awareness, critical analysis and logical integration of scientific facts. The conclusions of the literature review are logical and make a smooth transition to the next chapter of the dissertation work.

The aim of this dissertation is to determine the vitamin D and vitamin B12 status of pregnant women with normal and pathological pregnancies and to evaluate the role of their deficiency and/or insufficiency on the course of pregnancy and its outcome.

The goal is clearly and precisely formulated. **10 specific tasks** are also logically derived.

The material and method section presents the clinical contingent, comprising 259 pregnant women over the age of 18, with a singleton pregnancy, divided into three groups - healthy pregnant women (167 individuals), pregnant women with gestational diabetes (43 individuals) and pregnant women with preeclampsia (49 persons). The study was conducted in the DCC "St. Marina" - Varna, ACC MC - Varna, SBGAL "Prof. Dr. D. Stamatov" - Varna and MBAL "St. Anna" - Varna for the period from 02.07.2019 - 31.12.2021

Pregnant women fill out a questionnaire containing demographic data and information related to their lifestyle, previous pregnancies (if any) and obstetric outcomes, as well as family history. Information on the state of the mother and the fetus during pregnancy was taken and processed from the medical documentation of the OB-consulting offices and the indicated hospital medical facilities.

Information on the anthropometric parameters of the newborns after birth was obtained from the neonatology departments, the OB specialists or from the participants themselves. All pregnant

women underwent anthropometric tests - body weight, gestational weight gain (GWG), body mass index (BMI).

All newborns underwent anthropometric tests - height and weight. Biparietal diameter, abdominal circumference and thigh length bone are determined by ultrasound methods.

Between the 24th and 28th weeks of gestation, blood was taken for testing vitamin D, total vitamin B12, active vitamin B12, methylmalonic acid and insulin. In the same period, an oral glucose tolerance test (OGTT) was performed for the at-risk patients in order to diagnose gestational diabetes. Pregnant women with preeclampsia had their vitamin D and vitamin B12 status determined during their hospital stay.

The study of 25-hydroxy vitamin D3 25(OH)D and methylmalonic acid (MMA) was performed with a developed and validated liquid chromatographic method with mass-selective and UV detection.

Total vitamin B12 and insulin were determined according to the principle of direct chemiluminescence (CLIA) on an ACCESS 2 immunological analyzer.

Active vitamin B12 (Holotranscobalamin) was determined according to the principle of chemiluminescent immunoassay with microparticles (CMIA) on an immunological analyzer ARCHITECT.

Glucose was determined by the hexokinase method on a Mindray biochemical analyzer.

The laboratory methods used are modern and reliable. They make it possible to obtain reliable results corresponding to the generally accepted world standards for similar studies.

The obtained results were processed with modern statistical methods (IBM SPSS statistical package).

The results, a significant part of which have been published, are presented in 8 sections. They are logically systematized and well illustrated with appropriate tables and figures.

When looking at demographics, the mean age of the pregnant women was 29.84 years (with a range of 19 to 43 years). The percentage of women over 35 years of age in the groups with developed pregnancy complications is greater (20.38%) compared to that of healthy pregnant women (12.57%). The mean calculated BMI for all participants was 23.08 ± 3.93 (range: 15.24 to 44.98). In the group of healthy pregnant women, the value is 22.69 ± 3.69 , in pregnant women with GDM - 23.06 ± 4.91 , in women with preeclampsia - 24.25 ± 3.66 .

Gestational weight gain (GWG) in healthy pregnant women is 8.68 ± 4.78 kg, in pregnant women with GDM it is 9.69 ± 4.86 kg, and in pregnant women with preeclampsia - 13.91 ± 4.73 kg.

When looking for a relationship between seasonality and the development of pregnancy complications, no significant correlation dependence was demonstrated.

The anthropometric data on newborn children show that in healthy pregnant women the percentage of newborns weighing less than 2500 g is 7.2%, in patients with diabetes - 13.95%, in patients with preeclampsia - 33%.

When monitoring vitamin D status in pregnant women, the average value of 25(OH)D was 76.26 ± 38.27 nmol/l, the median - 69.15 nmol/l (range: 10.25 nmol/l to 204.23 nmol/l). The mean value of 25(OH)D was lowest in pregnant women with gestational diabetes (73.98 nmol/l), followed by those in healthy pregnant women (76.72 nmol/l) and in pregnant women with preeclampsia (76.85 nmol/l). No statistically significant difference was found in 25(OH)D results between the three study groups.

Vitamin D levels below the generally accepted optimal level of 80.00 nmol/l were found in 54.05% of pregnant women. Of these, 4.63% have an absolute deficiency, and 49.41% have a varying degree of deficiency. The highest frequency of absolute vitamin D deficiency was found

in pregnant women with preeclampsia (6.12%), while in the group of healthy pregnant women and in that of women with diabetes, the percentage of those with absolute deficiency was approximately the same (4.79% for healthy and 4.65% for women with diabetes. Vitamin D insufficiency was highest for the group with GDM (60.47%), followed by the group of women with preeclampsia (55.10%) and lowest in the group of healthy pregnant women (52.69%).

A statistically significant difference was found in the values for vitamin D in samples taken during the winter and summer semesters (winter semester - 71.40 ± 36.15 , summer - 85.30 ± 40.63 , $p < 0.01$). No statistically significant differences in the level of vitamin D were proven between the three main groups of pregnant women studied, distributed in the two halves of the year.

When distributing pregnant women according to body mass index, it was found that 71.43% were of normal body weight ($BMI < 25$) and 28.57% were overweight ($BMI > 25$). No statistically significant differences were found in serum vitamin D levels between the normal weight group and the overweight group.

Of pregnant women, 32.82% did not report taking vitamin D, while 67.18% took vitamin D as a pharmaceutical product and/or dietary supplements. The level of vitamin D in unsupplemented women was 56.05 ± 29.78 nmol/l, and in supplemented women 86.41 ± 38.50 nmol/l, $p < 0.0001$). Nearly 60% of supplemented women achieved optimal serum 25(OH)D levels, whereas only 21% of non-supplemented women achieved levels indicating sufficiency. Statistically significant differences were reported between the pregnant groups not only in terms of vitamin D intake, but also in terms of the dosage of units taken per day.

In the present study, 12.45% of the newborns were born prematurely (32 out of a total of 257 newborns). The complication rate was highest in the group of pregnant women with preeclampsia - 45.83% ($n=22$), followed by 6.98% ($n=3$) for pregnant women with GDM and 4.21% ($n=7$) in healthy pregnant women. The proportion of non-supplemented with vitamin D was higher among those who gave birth prematurely compared to those who gave birth at term (37.50% vs 32.44% respectively). The probability of having a low birth weight child was lower in those who were supplemented with an adequate dose of vitamin D (>600 IU/day) compared to those who were unsupplemented or supplemented with a low dose of vitamin D (<600 IU).

When examining the vitamin B12 status of pregnant women, a level of total vitamin B12 177.58 ± 90.24 pmol/l (median 429 pmol/l; range 76 - 782 pmol/l), of active vitamin B12 73.26 ± 53.25 pmol/l (median 273.70 pmol/l; range 16.40 - 531 pmol/l) and of methylmalonic acid 249.53 ± 154.73 nmol/l (median 584.34 nmol/l; range 40.94 - 1127.74 nmol/l).

Levels of total vitamin B12 above 250 pmol/l or vitamin sufficiency were found in only 13.51% of participants. Active vitamin B12 or sufficiency above 50 pmol/l was observed in 63.70% of pregnant women. Regarding the metabolic parameter MMA, serum concentrations above 300 nmol/l, proving vitamin B12 deficiency, were measured in 31.65% of the participants.

Higher serum concentrations of total and active vitamin B12 were measured in the serum of pregnant women who were sampled during the winter half-year.

Pregnant women with high BMI and those with normal BMI had similar mean but low levels of total and active vitamin B12 and similar mean but higher levels of the metabolic parameter MMA.

Of the pregnant women included in the current study, 34.75% did not report supplementation with the vitamin. A statistically significantly higher percentage of unsupplemented women and women receiving an inadequate amount of vitamin B12 was observed in women with gestational diabetes and preeclampsia.

Pregnant women supplemented with an optimal dose of vitamin B12 have the lowest probability of preterm birth.

In women who gave birth to children weighing less than 2500 g, the mean serum concentrations of the parameters total and active vitamin B12 were slightly lower, and the mean serum concentrations of the metabolite MMA, respectively, slightly higher, compared to those of women who gave birth to newborns of normal weight.

The obtained results cover the set goal and tasks of the work and show the ability of the dissertation student to build a scientific hypothesis and critical analysis of the obtained data.

The discussion shows the author's ability to objectively compare and contrast his data with world results.

Risk factors for the occurrence of pregnancy complications are considered - age, BMI, gestational weight gain, seasonality. Serum levels of 25(OH)D, total vitamin B12, active vitamin B12 and methylmalonic acid were assessed in the study cohort. The established data on the frequency of deficiency and/or insufficiency for the entire cohort, as well as for the three studied groups, were compared with data from studies concerning pregnant women from different regions of the world. An assessment of the place of Bulgaria was made in relation to the studied indicators. The dependence of vitamin D and vitamin B12 status on the intake of vitamin D and vitamin B12 as pharmaceutical products and/or nutritional supplements is discussed both for the whole cohort and in the three studied groups. The impact of the specified micronutrients in healthy pregnant women and women with complications both in relation to the occurrence of premature birth and in relation to the birth of a child with low birth weight is discussed.

The conclusions of the dissertation - 9 in number - are clearly and precisely defined. They derive from the results and correspond to the set goals and objectives.

Nine **contributions** are highlighted - 5 original and 4 applied.

For the first time in Bulgaria, a study was conducted to investigate the vitamin D status of pregnant women - healthy and with pathological complications of pregnancy, by examining the serum concentrations of 25(OH)D by means of high-performance liquid chromatography with mass spectrometric detection (LC-MS).

For the first time in our country, the vitamin B12 status of pregnant women is determined by three interrelated laboratory parameters - total vitamin B12, active B12 and methylmalonic acid, the latter determined by means of high-performance liquid chromatography with mass spectrometric detection (LC-MS).

For the first time in Bulgaria, a study was conducted to investigate the vitamin B12 status of pregnant women - healthy and with pathological complications of pregnancy

For the first time, the relationships between vitamin D and vitamin B12 status of pregnant women and the development of preeclampsia, gestational diabetes mellitus, preterm birth and low birth weight have been investigated.

Data were obtained on the vitamin D and vitamin B12 status of pregnant women in Northeastern Bulgaria, which until now were missing.

The seasonal dependence of serum concentrations of 25(OH)D was confirmed, which must be taken into account when analyzing the results.

I share the contributions of the work, which have both a scientific theoretical and a scientific applied nature. They are of high national and international scientific and practical value.

The publications in connection with the dissertation work are 3 in number and are in refereed medical journals. 6 reports from scientific forums are also presented.

The abstract meets the requirements. It reflects in a synthesized form the most essential moments of the dissertation work in all its sections.

In conclusion, based on the material presented to me, I believe that Dr. Monika Toshkova Todorova has acquired theoretical and methodical knowledge and professional skills, having performed the planned tasks accurately and systematically. I believe that the volume, content and relevance of the dissertation meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and I recommend the respected Scientific Jury to award Dr. Dr. Monika Toshkova Todorova the educational and scientific degree "Doctor" in professional direction 7.1 Medicine, scientific specialty "Clinical Laboratory"

12. 04. 2023.
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