

## REVIEW

Rewriter: prof. Yana Dimitrova Bocheva, MD, PhD  
Head of Department "Clinical Laboratory"  
to the Faculty of Medicine  
in Medical University "Prof. Dr. P. Stoyanov" – Varna

Subject: PhD thesis of Dr. Monika Toshkova Todorova, full-time doctoral student in the "Clinical Laboratory" doctoral program, conducted in the Department "Clinical Laboratory", Faculty of Medicine in Medical University "Prof. Dr. P. Stoyanov" – Varna for the acquisition of an educational and scientific degree "PhD" in the field of higher education 7. Health care and sports, professional direction 7.1. Medicine and Doctoral Program "Clinical Laboratory", with scientific supervisor Associate Professor Daniela Ivanova Gerova, MD, PhD

On the basis of Order No. R-109-159/24.02.2023 of the Deputy Rector "Career Development" of the Medical University "Prof. Dr. Paraskev Stoyanov" city of Varna and with the decision of the Scientific Jury on the basis of Protocol No. 1/09.03.2023 I have been assigned to prepare a review for a dissertation on the topic "Role of vitamin D and vitamin B12 in pregnant women and newborns".

The review is prepared according to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the Regulations for the Development of the Academic Staff at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

All stages of the doctoral studies have been observed, there is no change to the original topic and supervisor. Dr. Todorova has submitted all the necessary documents and materials according to the requirements of the procedure for acquiring the educational and scientific degree "PhD" according to the requirements of the Regulations for the Development of the Academic Staff at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

### ***Brief biographical details of the applicant***

Dr. Monika Toshkova Todorova was born in Ruse, where she graduated from "Baba Tonka" Mathematical High School. He graduated from Medical University "Prof. Dr. P. Stoyanov" - Varna in 2004. with a master's degree in Medicine.

She acquired a specialty in clinical laboratory in 2013. in the same educational institution and a master's degree in "Health Management" at VUARR-Plovdiv. For the period 2019-2023. she is enrolled and trained as a full-time doctoral student at the Department of Clinical

Laboratory in the doctoral program "Clinical Laboratory". The work experience of Dr. Monika Todorova, described in the presented resume, includes the period 2007-2023. During this period, she worked consecutively in the Central Clinical Laboratory at the UMBAL "St. Marina"-Varna /2007-2016/ as a resident, in Medical Center "Medica Ruse" OOD and in Diagnostic center 2-Ruse /IX.2016-VII.2017/ , in Diagnostic center "St. Marina"-Varna/from VIII.2017/, Medical Center "Ajibadem City Clinic"-Varna/since III.2019/. as a "Clinical Laboratory" specialist and in the Department of "Clinical Laboratory at the "Medical University" Prof. Dr. P. Stoyanov"-Varna as an assistant since II.2018. She quickly acquires the competence to carry out teaching activities and adequate professional skills to carry out her dissertation work. Fluent in English. She is a member of the Bulgarian Medical Union and the Bulgarian Society of Clinical Laboratory.

### ***Relevance of the topic***

The topic of the presented dissertation work has been selected carefully and according to the personal clinical interests of the candidate and the accumulated expertise and orientation of the scientific supervisor. Modern diagnostic medical specialties including clinical laboratory, have directed their efforts towards the validation of new laboratory, genetic and molecular markers, and in search of new projections of known parameters.

Pregnancy - normal or pathological, the outcome and birth of a healthy and viable fetus, as well as the reasons that led to complications or adverse outcomes for the mother and the newborn are and will be the subject of scientific research by various multidisciplinary teams. And although nutritional deficiencies and nutritional supplementation errors in this specific population group are more of a problem in developing countries and specific ethnic and minority populations in developed European countries, vitamin D and vitamin B12 deficiencies are being studied so intense that it is logical for a Bulgarian scientific team to formulate hypotheses and to build a research on a topic vitamin D and vitamin B12 status in pregnant women and newborns .

A large volume of data has been accumulated on the prevalence of insufficiency and deficiency of these two micronutrients among different target groups, including pregnant women. Information has been accumulated both on the adverse health effects and complications of pregnancy, as well as on short-term and long-term consequences for the health of the child and the mother as a result of the widespread insufficiency and deficiency of vitamin D and vitamin B12, and still in Bulgaria the status of vitamin D and vitamin B12 in pregnant women and newborns is not targeted and there is no health policy for follow-up and prevention of their restriction. A logical result of this information vacuum in our country at the moment are the scarce data and lack of Bulgarian scientific publications on the topic that motivated the research interest of the dissertation student and the scientific supervisor.

In this context, I consider the conceptual project of this dissertation presented to me for evaluation to be relevant, promising in published results, and contemporary classical in methodological execution.

### ***Characteristics, volume, structure, and content of the dissertation***

The dissertation consists of 178 pages, including: Introduction - 2 pages, Literature review - 51 pages, Aim and objectives - 2 pages, Materials and methods - 9 pages, Results - 53 pages, Discussion - 32 pages, Conclusions - 2 pages, Contributions - 2 pages, Publications - 1 page, References - 24 pages. The presentation includes 59 tables and 34 figures. The bibliography contains 350 sources, 4 of them are in Cyrillic. The latter includes contemporary sources, 72% of them are from the last 10 years.

The structure of the dissertation work is balanced and meets the modern formulations for the internal ratio of the parts of the dissertation work without theory and methodological excess with sufficient emphasis on the results and their interpretation. A clear style is demonstrated, with an excellent usage of highly specialized terminology.

**The literature review** is extensive, logical, well-structured and follows an identical style and sequence in the consideration of the two micronutrients. The text includes excessively detailed physiological and propaedeutic data on pregnancy as a physiological state, defines pathological pregnancy and adverse outcomes for the mother and newborn in the same style, and moves on to biosynthesis, metabolism and physiological role of the two indicators discussed with subsequent definition of needs, supplementation to maintain optimal levels of vitamins, effects on fetal development in conditions of insufficiency or deficiency. The cut-off values determining the vitamin D status are also examined and the need for a combination of the measurement of serum concentrations of total vitamin B12 with subsequent monitoring of methylmalonic acid levels is emphasized. The tabled recommendations for daily intake of the two micronutrients by age and physiological state are quite valuable. Current data on the prevalence of vitamin D and B12 deficiency in the general population are reported and its high incidence in pregnant women in specific geographic areas and among a number of ethnic minorities is discussed.

The review summarizes information on the analytical methods available in practice for the determination of Vitamin D and Vitamin B12, methylmalonic acid and homocysteine and discusses the analytical sensitivity, accuracy and reliability of the laboratory analyzes in a comparative plan. The latter, in my opinion, is expected by a dissertation work of a laboratory specialist and I consider the information presented to be insufficient. The main conclusion from the literature review is the availability of limited data on the vitamin D and B12 status of pregnant women, the frequency of their deficiency/insufficiency among this target group, as well as on the relationship between vitamin D and B12 deficiency and possible complications occurring during pregnancy for Bulgaria.

**The aim** is clearly formulated and is a logical consequence of the conclusions in the literature review, it corresponds to the title and is specified by the text of the tasks of the dissertation work.

There are 3 main tasks, two of which are semantic associations with 5 subtasks each, related to vitamin D and related to vitamin B12. They are derived logically from the formulated aim and are well defined.

The work is a prospective study with a great number of experimental targets and objectives, extending mainly in the field of interpretation of the obtained results for vitamin D and B12 status and the impact on pregnancy, the health of the newborn and the incidence of pregnancy complications.

### ***Materials and methods***

Dr. Todorova's dissertation is the result of long-term and ambitious research work on a scientific project supported by the "Science" Fund at MU-Varna, in which participate various preclinical and clinical specialists. The design of the study is prospective multicenter, performed in Diagnostic center "St. Marina" - Varna, Medical Center "Ajibadem City Clinic"-Varna - Varna, Specialized hospital in obstetrics-gynecology and neonatology for active treatment "Prof. Dr. D. Stamatov" - Varna and "St. Anna" University Hospital- Varna for the period 02.07.2019 - 31.12.2021 The characteristics of the participants - 259 pregnant women and the methods used to fulfill the set tasks and scientific aim, are presented clearly and completely by the PhD student. The work was carried out entirely according to the rules of good scientific practice, after permission was obtained from the Committee on Ethics of Scientific Research (KENI) at MU-Varna (protocol No. 84/27.06.2019). The analytical methods, some of which have been developed and validated for the needs of the scientific project, are precisely conducted, and described with attention to each of the stages of the laboratory study - standard pre-analytical rules are followed, pre-analytical sample preparation of the analyte and a perfectly described analytical stage of the chromatographic analysis with UV and mass-selective detection, incl. identification and quantitative analysis and validation procedure. The latter was conducted and described according to the requirements of the International Conference on Harmonization of Technical Requirements for the Registration of Medicinal Products for Human Use with included data on the standard validation parameters for both methods for the determination of 25-hydroxy vitamin D3 25(OH)D and methylmalonic acid. Statistical methods include descriptive, variance, correlation, non-parametric analysis and Student-Fisher parametric t-test and graphical analysis to visualize the results obtained.

### **Results**

In a total of 53 pages, Dr. Todorova describes in detail and consistently the implementation of each of the tasks, leading to the fulfillment of the formulated aim of her scientific work. 59 tables and 34 figures have been compiled, accompanied by an understandable text, and the applied statistical methods of analysis have been correctly selected and correctly interpreted.

**Task I:** The author collected, analyzed, and distributed the included patients into three groups: Group I - healthy pregnant women, Group II - pregnant women with gestational diabetes (GDM) and Group III - pregnant women with preeclampsia. Data from anthropometric measurements and screening for (GDM) are summarized and illustrated by figures and tables correlating with textual analysis and interpretations to present pregnant women's age, BMI calculated at the time of pregnancy, gestational weight gain and season as potential risk factors for the occurrence of adverse pregnancy complications – GDM, preeclampsia.

**Task II:** vitamin D status - the author consistently and very well presents the results of the study of the serum levels of 25(OH)D of the pregnant women included in the study. The vitamin D status was determined in the entire cohort of studied pregnant women and the distribution of cases with deficiency, insufficiency and sufficiency of the vitamin was presented, the influence of the body mass index and the season on the vitamin D status in the three studied groups was evaluated.

The dependence of the vitamin D status on the intake of vitamin D as a pharmaceutical product and/or nutritional supplements containing the vitamin in the three studied groups was investigated.

The results show a statistically significant difference in the distribution of pregnant women according to their vitamin D status depending on the intake of vitamin D preparations. The high rate of

lack of vitamin D intake in the group of pregnant women with preeclampsia is striking. In the analysis of the results, the tendency of pregnant women with high BMI to have a lower attitude towards supplementation is of interest. A significant but weak negative correlation between BMI and vitamin D intake was found, as well as a positive and significant correlation between vitamin D intake and serum levels of 25(OH)D.

When assessing the influence of vitamin D status on adverse pregnancy outcome regarding the newborn and the occurrence of preterm birth and low birth weight in healthy and pregnant women with pregnancy complications, a positive and moderately strong correlation was found between serum levels of 25(OH)D and duration of pregnancy in women with preeclampsia. The proportion of not-supplemented women was higher among women who gave birth prematurely compared to those who gave birth at term.

A positive and moderately strong correlation was found between serum levels of 25(OH)D and duration of pregnancy in women with preeclampsia, as well as between serum levels of 25(OH)D and intake of vitamin D preparations. When evaluating the effect of vitamin D status of pregnant women on the birth of a low birth weight (LBW) infant, it was found that the proportion of not-supplemented women who gave birth to a LBW child was three times greater than that of women who gave birth to a normal weight child. frequency distribution analysis by group for this adverse indicator shows that among healthy pregnant women and women with preeclampsia, the probability of giving birth to a low-birth-weight child is lower in those of them who are supplemented with an adequate dose of vitamin D compared to those not-supplemented or supplemented with low-dose vitamin D. From a detailed subgroup analysis of the mean serum 25(OH)D concentrations from the pooled results obtained in the two groups of women with pregnancy complications (preeclampsia and GDM) who gave birth to a normal-weight newborn and those who gave birth to a low-birth-weight child, it is striking, that they were 31.09% higher in women who gave birth to a normal-sized child compared to those who gave birth to a LBW child.

**Task III:** Vitamin B12 status—Serum levels of total vitamin B12, active vitamin B12, and methylmalonic acid and the incidence of vitamin B12 deficiency or insufficiency were determined. To determine the vitamin B12 status of the pregnant women included in the study, the algorithm proposed by Carmel was chosen. When comparing the three main groups of pregnant women regarding the average measured values of vitamin B12 indicators in the sample, it was detected that statistically significant differences were found only for the parameter total vitamin B12, as well as a tendency for statistically significant differences for methylmalonic acid (MMA). Higher measured values for the metabolic parameter MMA were found in pregnant women with preeclampsia and GDM. The influence of the season on the vitamin B12 status of the studied pregnant women was also investigated, as higher serum concentrations of total and active vitamin B12 were measured in the serum of the pregnant women in whom the sampling was performed during the winter semester. When evaluating the influence of body mass index on vitamin B12 status in the group of pregnant women with GDM, the mean measured serum concentrations of the parameters total and active vitamin B12 in overweight and obese participants ( $BMI \geq 25$ ) were slightly lower, and those of the metabolic parameter MMA, respectively, slightly higher, compared to the group of pregnant women with a normal BMI. The correlations are not statistically significant. The dependence of vitamin B12 status on the intake of nutritional supplements containing the vitamin was assessed in the three studied groups. Statistically significant differences in vitamin use were found between the three main groups of study participants. A

statistically significantly higher percentage of u not-supplemented women and women receiving an inadequate amount of vitamin B12 was observed in women with GDM and preeclampsia. There is evidence of an increased likelihood of lower or no intake in pregnant women who have developed pregnancy complications (GDM and pre-eclampsia). More than half (59.46%) of overweight and obese pregnant women have insufficient intake of vitamin B12. The results of this task also describe the role of vitamin B12 status on adverse pregnancy outcomes such as preterm birth, low birth weight in healthy pregnant women and women with pregnancy complications. The analysis found that pregnant women without or with insufficient supplementation of the vitamin were found among all three main groups. Significant statistical relationships between the duration of pregnancy and the parameters reflecting the vitamin B12 status of pregnant women from the compared groups were not found. A strong and positive relationship between the probability of delivering LBW infants with increasing total vitamin B12 deficiency was confirmed in women who developed preeclampsia. A weak but significant association between active vitamin B12 and the risk of having a low-birth-weight child was demonstrated in the group of healthy controls.

In the "**Discussion**" section of the dissertation, Dr. Monika Todorova makes an in-depth analysis of her results, comparing them with those of other scientific teams, respecting the sequence of the presented results, the logic of her presentation and keeping the guiding line of the set aim and tasks. This shows the good level of preparation of the PhD student, who demonstrates knowledge of Bulgarian and international resources on the problem under consideration. There is an openness and criticality towards some of the limitations of the research, which I personally admire.

I fully agree with the 19 conclusions, which reflect the results obtained. They are formulated clearly and specifically. They have the support and evidential material in the previous chapter of the dissertation.

The dissertation is completed with the definition of 5 contributions of an original nature and 6 of a practical-applied nature. I absolutely agree and believe that the results of this dissertation work will soon be cited in publications in national and foreign publications. I recommend a series of rapid publications with a view to wider publicity of the results obtained.

#### ***Publications and announcements on the subject***

A list of 3 full-text publications and 6 participations in national and international conferences is presented. Dr. Todorova is the first author of all full-text publications. The scientific activity of the dissertation student is in accordance with the Law on the Development of the Academic Staff of the Republic of Bulgaria and the rules for its application at the MU-Varna.

#### ***Conclusion***

The dissertation work of Ph.D. student Monika Toshkova Todorova is essentially an in-depth scientific development with a practical orientation. The topic is current, interesting, and challenging. The applied methods are adequate to achieve the set aim and tasks, the results are significant, and the conclusions are logical and correctly drawn. Contributions are original and practical. Dr. Todorova is an excellent laboratory specialist and has put a lot of work, patience, and persistence into the development of this work.

The dissertation work on the topic "Role of vitamin D and vitamin B12 in pregnant women and newborns" with author Dr. Monika Toshkova Todorova covers the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the relevant regulations of the Medical University "Prof. Dr. P. Stoyanov" - Varna.

Based on the presentation, I give a positive assessment of her dissertation work and recommend the honorable jury to award Ms. Monika Toshkova Todorova the educational and scientific degree "DOCTOR IN MEDICINE".

26.04.2023

Reviewer:.....

A handwritten signature in blue ink, appearing to be 'Yana Bocheva', written over a dotted line.

/prof.Yana Bocheva, MD, PhD./