

## **REVIEW**

**by Assoc. Prof. Milena Ivanova Belcheva, MD, PhD**

**Department of Pediatrics, MU "Prof. Dr. Paraskev Stoyanov", Varna**

On a dissertation for the award of the educational and scientific degree "Doctor"

**Field of higher education: 7. "Health care and sports"**

**Professional direction: 7.1 Medicine**

**Doctoral program: Pediatrics**

**Author:** Dr. Kristina Ivaylova Petrova, Department of Pediatrics, Faculty of Medicine, Medical university-Varna

**Topic:** *Assessment of oxidative stress and early vascular damage in children and young adults with Beta-thalassemia major*

**Scientific supervisors:** Prof. Valeriya Ignatova Kaleva, MD, PhD

Assoc. Prof. Maria Stoyanova Dimova-Mileva, MD, PhD

### **General presentation of the procedure**

By order No. RD-109-139/05.04.2024 of Prof. Dimitar Raykov, MD, PhD, Rector of Medical University "Prof. Dr. Paraskev Stoyanov" Varna, based on the decision of the Faculty Council of the Faculty of Medicine (Protocol No. 19/01.04.2024) I was elected as an internal member of a scientific jury evaluating the dissertation work of Dr. Kristina Ivaylova Petrova on the topic: *Assessment of oxidative stress and early vascular damage in children and young adults with Beta-thalassemia major*. Based on the Protocol No. 1/15.04.2024 from the first meeting of the scientific jury, I have been appointed to prepare a review.

The set of materials provided by the doctoral candidate for the procedure for awarding the title "Doctor" is complete and available as an electronic version and in paper form according to the Regulations for the Development of the Academic Staff of Medical university of Varna, as well as the Law on the Development of the Academic Staff in the Republic of Bulgaria.

### **Brief biographical data of the PhD student**

In 2013 Dr. Kristina Ivaylova Petrova graduated from Medical university of Varna. Until present she has been working in the Pediatric Clinical Hematology and Oncology clinic in University hospital "Sveta Marina", Varna as a resident. From October 2019 to the present, is an assistant professor in the Department of Pediatrics at the Medical university of Varna. In 2020, she was enrolled as a full-time doctoral student in the Pediatrics doctoral program. She has acquired specialties in pediatric clinical hematology and oncology and in pediatrics.

Dr. Kristina Petrova has been participating in national and international scientific forums. Her scientific interests are in the field of hereditary anemias and malignant oncohematological diseases. She is a member of the Bulgarian Medical Union, the National

Association of Pediatric Hematology and Oncology, the Bulgarian Pediatric Association and SIOP Europe (European Society for Pediatric Oncology).

### **Relevance and importance of the topic**

The modern complex care for patients with beta-thalassemia major results in change in the clinical evolution, the spectrum of complications and the prognosis of the disease. Due to the increasing life expectancy, the clinical focus gradually shifts from heart failure, a major cause of mortality in the past, to vascular pathology conferring significant cardiovascular risk. In beta-thalassemia major patients there is a combination of multiple risk factors leading to vascular damage. To the oxidative stress caused by chronic anemia and iron overload are added the effects of atherogenic dyslipidemia and hormonal deficiencies typical for the disease. All of these are superimposed on the natural aging process and are potentiated by unfavorable behavioral stereotypes.

Dr. Petrova analyzes biomarkers of oxidative stress and indicators of early vascular damage in young patients with beta-thalassemia major, which are reliable predictors of cardiovascular risk and give an opportunity for early intervention ensuring long-term survival in good health and improving the quality of life for individuals from this high-risk group.

I find the chosen topic of the dissertation important, relevant and extremely useful for clinical practice.

### **Characterization and evaluation of the dissertation work**

The dissertation is presented in 102 standard pages (without the bibliography). Illustrated with 36 tables and 21 figures. The figures are of good quality and sufficiently informative to demonstrate the established statistical significances. It is structured as follows: literature review - 32 pages; purpose and tasks - 1 page; material and methods - 7 pages; own studies and discussion of the obtained results - 49 pages; conclusions - 2 pages; contributions - 1 page; list of publications and participation in scientific forums related to the dissertation - 1 page; bibliographic reference - 21 pages. An acceptable ratio between the individual sections of the scientific work has been observed. 358 adequately selected literary sources are cited.

#### *Literature review*

The literature review is comprehensive and shows excellent knowledge of the scientific issues. The pathophysiology of oxidative stress, the toxic effects of reactive oxygen species, in particular lipid peroxidation, as well as the methods for its evaluation are presented in detail. The role of oxidative stress in the development of endothelial dysfunction and the resulting continuum of altered vascular elasticity, arterial stiffness and atherosclerosis is highlighted. The problem of oxidative stress in patients with beta-thalassemia major is presented in detail and in depth, in which, in addition to the general effect of free radicals, the oxidative effect of free iron accumulated in the body is superimposed. Dr. Petrova presents numerous data obtained from long-standing scientific research by teams from all over the world, which prove the presence of a distinctive lipid profile, an early manifestation of arterial stiffness, an early development of atherosclerosis and the resulting high cardiovascular risk in patients with beta - thalassemia major. The manner of execution, the location and the significance of some modern tools for the assessment of arterial stiffness in this high-risk target group are discussed in detail.



The literature review ends with a justified conclusion about the need to deepen the studies assessing the predictive role of the abnormal lipid profile and oxidative stress for the development of early atherosclerosis in patients with beta-thalassemia major and the need to validate pulse wave velocity  $\beta$  (PWV $\beta$ ) as a local parameter of carotid arterial stiffness.

The *aim* of Dr. Petrova is to identify the presence of early vascular damage by studying arterial stiffness of peripheral vessels and to study its correlations with markers of oxidative stress, lipid profile indicators and lipid indices in children and young adults with beta-thalassemia major. Ten specific *tasks* are set, which are logical and clearly formulated.

### *Materials and methods*

The study is performed as a case-control type. The selection of participants was based on precisely formulated inclusion and exclusion criteria. Patients treated and followed up at the Expert Center for Coagulopathies and Rare Anemias at University hospital "Sveta Marina", Varna, were included. A total of 78 children and young adults were studied, of which 38 patients (age range 5-44 years, mean age 25 years) and 40 age- and sex-matched healthy controls. The analyzed contingent of participants is sufficient in number and allows statistical analysis.

The studies were performed prospectively and included:

- Anthropometric parameters: weight, height, BMI, BSA
- Measurement of heart rate, systolic, diastolic, mean arterial and pulse pressure
- Laboratory tests: blood count with differential count, lipid profile, serum ferritin, serum iron, C-reactive protein, malondialdehyde
- Lipid profile and lipid indices and ratios: total cholesterol, LDL- and HDL-cholesterol, triglycerides, Castelli risk index I (CRI-I), Castelli risk index II (CRI-II), plasma atherogenic index (AIP), atherogenic factor (AtC), non-HDL-cholesterol
- Instrumental studies: echographic examination by echo-tracking of the two common carotid arteries with calculation of arterial stiffness indicators:  $\beta$ -stiffness index, arterial compliance (AC), pulse wave velocity  $\beta$  (PWV $\beta$ ), augmentation index (AIx) and pressure-strain elastic modulus (Ep)
- Evaluation of harmful habits, symptoms of cardiovascular disease and antihypertensive therapy by means of a questionnaire

Research methods are described in detail and consistently and give a clear idea of the approach to the problem. They allow obtaining real results and solving the set tasks. A very strong point of the work is the independent echographic examination by echo-tracking of the carotid arteries by Dr. Petrova, for which she was prepared by targeted and certified training.

Methods for statistical data processing are presented in detail. SPSS Windows version 25 software package was used.

### *Results and discussion*

The results of the study are clearly presented and separated into three sub-sections. They are well illustrated with tables and graphics. In the first subsection, the demographic and anthropometric characteristics of the participants, the type of chelation therapy in patients with beta-thalassemia and the proportion of those undergoing splenectomy among them were analyzed. The selection of participants in the two studied groups - patients and controls - was



well balanced, with no significant differences in age, gender, height, weight, BSA and BMI. The majority of patients (74%) underwent chelation therapy with Deferasirox; 34% were splenectomized. The proportion of smokers in both groups is comparable.

The second subsection presents the results of the comparative analysis of the studied parameters in the patients with beta-thalassemia major and the control group. They are presented in a sequence corresponding to the set tasks.

According to task 1, the doctoral candidate found significantly higher diastolic, mean arterial pressure and a higher heart rate in the patients.

According to task 2, significantly lower values of hemoglobin, erythrocytes and hematocrit, as well as significantly higher serum iron, triglycerides and ferritin were demonstrated in participants with beta-thalassemia major compared to the control group.

According to task 3, Dr. Petrova proved that in patients compared to their healthy controls a distinctive lipid profile with significantly lower total cholesterol, LDL-cholesterol and HDL-cholesterol and high triglycerides is present, while the average values of the indicators remain within reference limits. Mean values of lipid indices were higher in patients compared to healthy controls, the difference being significant for Castelli Risk Index I and Atherogenic Index of Plasma.

According to task 4, the doctoral candidate found a relatively higher, but comparable to that in healthy individuals, serum concentration of malondialdehyde in patients with beta-thalassemia major.

According to task 5, the analysis of local arterial stiffness by echo-tracking methodology demonstrated a significantly higher arterial compliance of the right common carotid artery in the patient group.

The third subsection presents the results of the correlation analysis of the studied parameters in the group of patients with beta-thalassemia major.

According to task 6, the doctoral candidate proved a strong positive correlation of serum ferritin with total and LDL-cholesterol, serum triglycerides and lipid indices CRI-I, CRI-II, AtC and non-HDL-cholesterol.

For task 7, the analysis showed a strong positive correlation between hemoglobin values and total and LDL-cholesterol, as well as a moderate negative correlation with the lipid index AIP in patients with beta-thalassemia major.

According to task 8, Dr. Petrova found a moderate to strong positive correlation between age and the values of PWV $\beta$  and  $\beta$ -stiffness of the carotid arteries, as well as significantly higher values of vascular stiffness indicators in patients over 26 years of age compared to - young patients. A positive correlation of the arterial stiffness indicators in the female sex has also been proven. A moderate positive correlation was found between  $\beta$ -stiffness and PWV $\beta$  and serum triglycerides, as well as a moderate to strong positive relationship between all arterial stiffness parameters of both carotid arteries and all investigated lipid indices/ratios without AIP.

According to task 9, the correlation analysis did not reveal a significant relationship between the parameters of arterial stiffness and the serum levels of ferritin and malondialdehyde.

According to task 10, the doctoral candidate found a significant positive correlation between previous splenectomy and the levels of total, LDL-cholesterol and non-HDL, and a moderate positive correlation with CRI-II. A moderate positive correlation between performed splenectomy and the indicators of arterial stiffness is also outlined.

The discussion is interwoven into the own research with commentary on each of the results obtained. In it, Dr. Petrova shows a good knowledge of the literature sources and upgrades the information regarding risk factors and the pathogenesis of vascular damage in patients with beta-thalassemia major. Comparisons have been made with the results of numerous studies concerning all analyzed indicators. An own interpretation was made of the differences and similarities found between the studies. Recommendations are made for the clinical follow-up of patients.

The conclusions of the dissertation work are ten and summarize the results of the ten set tasks. Attention should be paid to the statement - "credible differences", "credible correlations" should be replaced with "statistically significant" and "significant" differences, etc.

### **Evaluation of the contributions of the dissertation work**

The presented contributions are original, scientific-practical and confirmatory in nature. In essence, the dissertation is the first study in Bulgaria of carotid artery stiffness in patients with beta-thalassemia major using echo-tracking methodology and correlation of early vascular damage with lipid profiles and indices, iron overload and oxidative stress. The obtained results have scientific and applied significance, offering clinical tools for monitoring patients from their early age with the aim of timely therapeutic intervention.

### **Bibliography**

The bibliography includes 358 literary sources, of which 12 are in Cyrillic and 346 are in Latin. Most of them are contemporary, 20% were published in the last 5 years and reflect the current achievements in the field of scientific topics.

### **Assessment of dissertation publications**

Dr. Petrova presents 3 full-text publications related to the dissertation, two of which are in a refereed edition. She is the first author of two of the publications. In addition, it also presents two participations in national scientific conferences on the subject of the dissertation work.

### **Abstract**

The abstract is presented in 71 pages and structurally follows the content of the dissertation. It covers the individual sections in their entirety, except for the literature review, and includes all tables and figures with results. The abstract presents in full the aims and tasks, the methodology, the results of the own research and their discussion, the conclusions and contributions of the scientific research.



### Critical remarks

I believe that the work would have benefited from following the classical structure of a dissertation paper with the discussion of the results obtained in a separate section.

In a study of this type, it is appropriate to use regression analysis to more accurately determine the relationships between the individual analyzed parameters.

### CONCLUSION

The dissertation contains original and applied scientific results and meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations of the MU "Prof. Dr. Paraskev Stoyanov" - Varna for awarding the scientific and educational degree "Doctor".

Dr. Petrova's dissertation reflects several years of in-depth work - carefully prepared, precisely conducted and correctly analyzed. It is an original development of the doctoral candidate and demonstrates her profound theoretical knowledge and professional skills in the scientific specialty of Pediatrics, as well as her qualities and capacity to independently conduct scientific research.

I give my positive evaluation for the dissertation work on the topic " Assessment of oxidative stress and early vascular damage in children and young adults with Beta-thalassemia major" and propose to the esteemed scientific jury to vote "for" the award of the scientific and educational degree "Doctor" to Dr. Kristina Ivaylova Petrova.

18.06.2024

Reviewer:

Assoc. Prof. Milena Belcheva, MD, PhD

Заличено на основание чл. 5,  
§1, б. „В“ от Регламент (ЕС)  
2016/679