OPINION

of a dissertation for the acquisition of an educational and scientific degree

"doctor" in the scientific specialty "Pediatric "

on the topic: **"Influence of oxidative stress on early vascular damage in children and young adults with Beta-thalassemia major"**

Author: **Dr. Kristina Ivaylova Petrova**

Research supervisor: **Prof. Valeriia Ignatova Kaleva, MD**

Associate **Prof. Maria Stoyanova Dimova-Mileva, MD**

Reviewer: **Assoc. prof. Dr. Petar Shivachev, MD**

Department of "Pediatrics",

MU "Prof. Dr. Paraskev Stoyanov" Varna

This review was prepared according to Order No. P-109-139/05.04.2024 of Prof. Dr. Dimitar Raykov, MD, rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna and on the basis of the materials presented by the doctoral student: dissertation, abstract, report on the contributions and publications on the topic of the dissertation work.

1. Relevance of the problem developed in the dissertation in scientific and applied scientific attitude

The thesis proposed for review concerns an area that is undoubtedly debatable, and the problem is significant and insufficiently well developed on a global and national scale, especially in a practical-applied aspect. In this sense, the choice of topic is relevant and necessary.

Beta-thalassemia (BTM) is a hereditary hemoglobinopathy with damage to the beta-globin gene, reduced or absent synthesis of β-globin chains, oxidative damage to erythrocyte membranes from the formed unstable tetrameric aggregates, and premature hemolysis. The oxidative effect of free iron accumulated in the body as a result of regular hemotransfusions is also important. Lipid peroxidation is a major molecular mechanism of free-radical toxicity, and malondialdehyde (MDA) formed is a preferred marker for oxidative stress assessment. The resulting endothelial dysfunction and increased arterial stiffness are important precursors of atherosclerosis. Doppler ultrasonography of carotid arteries (CA) can early identify subclinical atherosclerosis.

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In this regard, it can be concluded that the topic of the dissertation is successfully chosen, current, with scientific significance and great practical application. In developing it, the dissertation student shows good theoretical preparation and analytical abilities. The dissertation is written in scientific language, with a very good style.

2. Degree of knowledge of the state of the problem and creativity interpretation of literature

The doctoral student has used a total of 358 literary publications, of which 21 are in Cyrillic and 337 are in Latin. Literary sources have been used in good faith and correctly, and some of them have been cited in the text of the dissertation, which makes it possible to highlight the personal contribution of the doctoral student. The bibliographic reference is sufficiently varied and rich to carry out an in-depth scientific study of the subject. The choice of scientific publications, the style and analytical nature of the dissertation work, allow us to summarize that the doctoral student knows well the achievements of science in the field he studies, systematizes known scientific achievements, synthesizes scientific theses, highlights and formulates unsolved problems.

3. Brief analytical description of the dissertation work

The dissertation submitted for review has a volume of 137 pages and is structured as follows: introduction - 2 pages, literature review - 34 pages, aim and objectives of the research - 1 page, material and methods - 7 pages, results and discussion - 50 pages, conclusions - 2 pages, conclusion - 1 page, self-assessment of the contributions of the dissertation work - 1 page, bibliography - 12 pages, appendices - 2 pages, abbreviations used - 1 page. The content of the chapters is divided into separate paragraphs, and at the end specific conclusions are drawn, which present the results of the research, summaries and assessments of the issues under consideration.

The main text contains 21 figures and 36 tables, which are appropriately formatted. The structure of the dissertation is classic and meets the requirements.

In **the introduction**, the doctoral student argues the relevance and importance of the topic and the research problem (pp. 5-6). The purpose and tasks of the research are formulated

(p. 41-42), the research object and methodology are defined (p. 43-48), as well as the statistical methods used (p. 48-49).

The object and subject of the dissertation work are correctly specified. A total of 78 participants were included, of which 38 children and young adults with BTM and 40 sex- and age-matched healthy controls, who met the inclusion and exclusion criteria.

Research objective – to identify the presence of early vascular damage by examining arterial stiffness of peripheral vessels and to study its correlations with some markers of oxidative stress, lipid profile indicators and lipid indices in children and young adults with beta-thalassemia major, is correctly formulated and corresponds to the real achievements shown in the dissertation work.

The tasks of the dissertation work are precisely and comprehensively defined, related to:

1. Comparative evaluation of some baseline hemodynamic parameters (heart rate, arterial pressure and pulse pressure) in patients with BTM and healthy controls.
2. Comparative evaluation of some hematological parameters (Hb, Ery and Hct) and indicators of iron overload (serum ferritin) in BTM patients and healthy controls.
3. Analysis of indicators of lipid metabolism and some atherogenic indices, as markers of cardiovascular risk, in patients with BTM and in healthy controls.
4. Determination of serum concentrations of MDA in patients with BTM and comparison with those of healthy controls
5. Measurement and comparison of the local arterial stiffness of the two CA by echo-tracking (ET) in patients with BTM and healthy controls.

6. Investigation of correlations between serum ferritin levels and indicators of lipid metabolism and some atherogenic lipid indices in patients with BTM.
7. Investigation of correlations between hemoglobin values and lipid profile indicators and lipid indices.
8. Study of the correlations of the ET parameters of the two CA with sex, age, indicators of lipid exchange and atherogenic lipid indices in patients with BTM.
9. Investigation of the correlations of the ET parameters of the two CA with the levels of serum ferritin and MDA in patients with BTM.
10. Study of correlations between splenectomy and ET parameters of both CA, indicators of lipid exchange and atherogenic lipid indices in patients with BTM.

Results and discussion. The obtained results, divided into three parts, are presented by the doctoral student systematically, according to the tasks, well illustrated with tables and figures, followed by a discussion.

1. **General characteristics of the study participants.** Anthropometric and demographic indicators were analyzed, such as age, gender, weight, height, body surface area, BMI, smoking, chelation therapy, splenectomy. No significant differences were found between the groups of BTM patients and healthy controls.
2. **Comparative analysis of the obtained results in patients with BTM and the control group.** Hemodynamic parameters were compared. As expected in patients with BTM from the ***hematological parameters***, the mean values of Hb, Ery and Hct were significantly lower, and those of serum iron and serum ferritin significantly higher. In the ***lipid profile*** of BTM patients, total cholesterol, LDL-cholesterol, and HDL-cholesterol values were lower and triglyceride values were higher compared to the control group, without statistically significant differences. Regarding ***lipid indices***, significance was found only for CRI-I and AIP, which was also reported by other authors. The difference in terms of ***MDA*** between the studied patients and the control group is also insignificant, which the doctoral student associates with the missed opportunity for a comparative study of the serum levels of antioxidant vitamins. The analysis of the ***echo-tracking (ET)*** indicators of the right common CA found a borderline level of significance only in AC (R), while for the left common CA did not find statistically significant differences.
3. **Correlation dependences of the results in patients with BTM.** Strong positive correlations of serum ***ferritin*** with a large number of parameters of ***lipid metabolism and lipid indices*** such as - total cholesterol, LDL-cholesterol, triglycerides, CRI-I, CRI-II and AtC have been demonstrated. A similar dependence in BTM patients has been established by many other authors. Correlation analysis of ***hemoglobin*** values and ***lipid profile*** indicators revealed a strong positive relationship with total cholesterol and LDL-cholesterol, a moderate positive relationship with HDL-cholesterol values and a moderate negative relationship with AIP values. A similar dependence has been found in other studies. Lower pre-transfusion hemoglobin levels are associated with more severe dyslipidemia, meaning that ineffective erythropoiesis is not suppressed and this leads to higher levels of oxidative stress and increased dyslipidemia. These results confirm the need to maintain pretransfusion hemoglobin levels above 95 g/L as recommended by the International Thalassemia Federation. Regarding ***ET*** to assess the arterial stiffness of the

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common CA, a moderate positive correlation of age with PWVβ values (R), a strong positive correlation with PWVβ (L), a moderate positive correlation with β-stiffness was observed in the BTM patient group (R) and a strong positive correlation with β-stiffness (L). When comparing these indicators in patients under and over 26 years of age, higher values were also found at older ages, which confirms data published in the literature that changes in arterial elasticity begin at a young age and increase with increasing age. Strong positive correlations of β-stiffness of both CA and of PWVβ of the left CA with gender were also demonstrated, more pronounced in women. Regarding the lipid parameters related to the development of atherosclerosis and vascular stiffness, statistically significant positive correlations of PWVβ and β-stiffness of both CA with CRI-I, CRI-II, AtC and triglycerides were demonstrated. The values of the β-stiffness parameter, measured on the left CA, also correlate positively with the mean values of total cholesterol. The study did not demonstrate a statistically significant correlation between ET indicators of arterial stiffness and laboratory values of MDA. Regarding serum ferritin, a statistically significant positive correlation was found only with the β-stiffness index for the right CA. A possible reason for the lack of correlations between these parameters is the small group of patients studied. Splenectomy correlated strongly positively with LDL-cholesterol and total cholesterol values. A strong positive relationship was also established with the levels of non-HDL-cholesterol, and a moderate positive relationship with the levels of CRI-II. Regarding arterial stiffness indicators, a moderate positive relationship was found with β-stiffness (L), Ep (L) and PWVβ (L) values. The results obtained are consistent with those of other studies and based on their significance, the PhD student assumes that the risk of CVD is greater in splenectomized BTM patients.

Based on the obtained results and literature data, Dr. Petrova logically formulates ten succinctly presented **conclusions**.

The abstract is compiled according to the requirements and correctly reflects all the main parts of the dissertation. In terms of content, the contributions indicated in the abstract reflect objectively the achievements of the doctoral student.

On the problems treated in the dissertation, PhD student Dr. Kristina Petrova has made **three publications** in Bulgarian publications and two participations in scientific forums.

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5. In children and young adults, there is a positive correlation between lipid indices and arterial stiffness indicators.
6. Splenectomy correlates with more severe dyslipidemia and arterial stiffness.
7. Identification of subclinical vascular damage is a potential approach for screening and prevention of cardiovascular complications in patients with BTM.

5. Publications on the dissertation work

Three publications of the doctoral student on the dissertation are indicated, which are sufficient and present the main points of the dissertation. It is clear that the PhD student's ideas and scientific achievements have received public recognition and are being used by the scientific community.

CONCLUSION

The dissertation proposed for review on the topic **"Influence of oxidative stress on early vascular damage in children and young adults with Beta-thalassemia major"** is the personal work of PhD student **Dr. Kristina Petrova**. The abstract is correctly composed, such as development, layout and presentation of scientific achievements and contributions. The publications correspond to the topic and content of the dissertation work. The dissertation research was developed in accordance with the requirements for the acquisition of the educational and scientific degree "doctor" of ZRASRB, and the Regulations for its application. Dissertation work has a completely finished form. It demonstrates current scientific and applied achievements, which represent a contribution to the practical solution of a wide range of problems related to patients with beta-thalassemia major. There are all the necessary grounds for me to categorically declare my positive assessment of Dr. Kristina Petrova's dissertation research.

In conclusion, I strongly suggest to the respected scientific jury to award Dr. Kristina Petrova the educational and scientific degree "doctor" in the scientific specialty "Pediatric ".

12.06.2024 г.

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4. Summary of strengths of the dissertation work

1. Дисертацията третира актуална и значима проблематика. The analysis of the pathophysiological chain - oxidative stress, endothelial dysfunction, altered vascular elasticity, enables the stratification of cardiovascular risk in patients with BTM, by evaluating lipid profiles/indices and echo-tracking indicators of the carotid arteries.

2. The dissertation is very informative and demonstrates the author's rich awareness of the issues presented in the analysis. The logical sequence of the exposition allows the author to argue his conceptual thesis.

3. In the introduction, the topicality of the topic is adequately commented, the research problem and the thesis of the study are formulated. The subject, object, purpose and tasks of the dissertation research are correctly defined.

4. The PhD student knows the problem in depth. The dissertation is based on a wide range of researched scientific sources. The author demonstrates analyticity and conceptuality.

5. The doctoral student shows indisputable research skills - he knows the subject matter very well, he highlights the unsolved problems of the researched issue.

6. The results achieved in the course of the dissertation research have been practically approved.

5. Scientific, scientific-applied and contributions with a confirmatory character

The dissertation research has scientific and scientific-applied contributions in the following directions:

1. For the first time in Bulgaria, in patients with BTM, the stiffness of the carotid arteries was measured using the echo-tracking methodology and the lipid profile and indices were evaluated, analyzing their relationships with the indicators of iron overload, oxidative stress and arterial stiffness.
2. Calculation of lipid indices is a potential clinical tool for cardiovascular risk assessment in patients with BTM.
3. Determining the stiffness of the carotid arteries using the echo-tracking technique is an accessible, non-invasive and useful method for assessing vascular health in children and young adults and can be included in the algorithm for monitoring patients with BTM.
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