

OPINION

Subject: dissertation work for awarding the educational and scientific degree "PHILOSOPHY DOCTOR" on the topic: "Possibilities of some echocardiographic techniques and microRNAs for detecting subclinical myocardial damage in children and young adults with beta-thalassemia major"

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Prepared the opinion: Assoc. Prof. Lachezar Radoslavov Marinov, MD, PhD, pediatric cardiologist, internal member of the Scientific Jury appointed by order of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna № R-109-187/15.03.2023.

At the first absent meeting of the Scientific Jury, I have been appointed to prepare a opinion.

The opinion has been prepared in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Application of the ZRASRB (PRZRAS) and the Regulations for the Terms and Procedures for Acquiring Scientific Degrees and Holding Academic Positions of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

The presented set of materials on paper/electronic media is in accordance with the requirements of ZRASRB, PPZRASRB, the regulations and the procedure for acquiring the scientific and educational degree "doctor" and the regulations of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

I do not detect plagiarism when reviewing the dissertation, abstract, and dissertation-related publications submitted to me for review.

I declare the absence of a potential conflict of interest from my participation in the scientific jury.

Brief biographical data

Dr. Kalina Ganeva graduated "Medicine" in 2012 at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. From 2013 to 2017 he specialized in "Pediatric Cardiology" at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. He acquired the specialty "Pediatric Cardiology", effective from 01.01.2018, certificate of recognized specialty No. 3891. He acquired a professional qualification for "Highly specialized activity in non-invasive functional cardiology diagnostics" from 11.03.2019, certificate No. 000525. From 2018 to 2020, he specialized in "Pediatrics" at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. She acquired the specialty "Pediatrics" as of 01.06.2021, certificate of recognized specialty No. 4653. From 01.02.2019 she was enrolled as a doctoral student in the regular form of study in the doctoral program "Pediatrics" at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

Very good level of command of the English language.

Member of the Bulgarian Medical Union, Bulgarian Pediatric Association, Society of Cardiologists in Bulgaria, European Society of Cardiology (ESC), European Association of Pediatric Cardiology (AEPC).

Relevance of the topic

Thalassemias are a group of inherited diseases characterized by reduced or absent production of the normal globin chains of hemoglobin. Beta-thalassemia major (BTM) belongs to the transfusion-dependent forms of thalassemia, which require regular lifelong hemotransfusions, without which a number of complications would occur. Over time, iron overload occurs from developing secondary hemochromatosis. The free form of iron, which is highly toxic, is deposited in the internal organs, mainly affecting the heart, liver and endocrine glands, causing a number of disorders such as heart failure, liver cirrhosis, endocrine disorders. Regular and adequate chelation therapy provides normal quality and life expectancy, but cardiac complications are still a leading cause of morbidity and mortality. There are reports of very early iron deposition in the myocardium, as early as 7 years of age, mainly with limited access to chelation therapy. The T2* magnetic resonance technique is considered the main method for assessing iron accumulation in the myocardium, but the method is expensive and not always possible. New echocardiographic methods and techniques for evaluation and follow-up of cardiac function and myocardial deformation, such as strain, strain-rate, torsion and twist in children are more acceptable, economically advantageous and easily feasible.

In recent years, microRNAs have attracted the attention of researchers and clinicians as potential biomarkers for various pathological conditions. Certain microRNAs (microRNA-1, microRNA-21, microRNA-29, microRNA-30 and microRNA-150) are known to be involved in the pathogenesis and progression of heart failure (HF), myocyte hypertrophy, apoptosis, interstitial fibrosis and cardiac remodeling. Complications with marked cardiac damage occur in the most active years of the patients' lives, and iron deposition is very likely to have started in childhood.

In Bulgaria, until now, no systematic studies have been carried out to assess cardiac function, myocardial deformation in children and young adults with BTM. The study of microRNAs is a new, innovative method to evaluate their role in the pathogenesis and progression of cardiac damage.

The dissertation work of Dr. Kalina Ganeva is relevant and essential for care and proactive behavior in this group of patients in Bulgaria.

Structure of the dissertation

The dissertation work of Dr. Kalina Ganeva is written on 130 pages, illustrated with 39 figures, 14 tables and 2 appendices. The bibliography includes 147 literary sources, of which 6 are in Cyrillic and 141 are in Latin. Most of the cited sources are from the last 10 years, with more than 20% from the last 5 years.

It is written in the literary Bulgarian language, well structured in accordance with the accepted standards for the preparation of a scientific work for the acquisition of a scientific and educational degree "Doctor". Contains the following pages: Title page – 1 page; Contents – 2 pages; Abbreviations used – 1 page; Introduction - 2 pages; Literature review - 29 pages; Purpose and tasks - 1 page; Material and methods - 12 pages; Results and discussion - 47 pages;

Conclusions - 1 page; Conclusion - 2 pages; Contributions - 1 page; Publications and participations related to the dissertation work - 1 page; Appendices – 12 pages; Bibliography - 16 pages.

The proportional distribution between the individual sections is respected.

The introduction is on 2 pages and points to the essence of the scientific work.

Review of the literature

Presented in 49 pages, extremely detailed and informative. It is written in literary Bulgarian. The dissertation student demonstrates a skillful handling of scientific terminology, a good awareness of the epidemiology, clinical presentation of BTM, pathophysiology, iron metabolism, monitoring of its level in the human body, the involvement of the heart - hemodynamic changes, iron-induced heart damage and its consequences complications. A special place in the review is devoted to modern non-invasive methods for early assessment of iron deposition in the myocardium in patients with BTM - MRI T2* examination and Echocardiography. In recent years, new echocardiographic techniques have been developed to assess myocardial deformation, such as strain, strain-rate, torsion and twist.

The main life-limiting cardiac complication in BTM patients is thalassemic cardiomyopathy (TCMP) with/without HF and heart rhythm disturbances. Arrhythmias and sudden cardiac death may occur even in the absence of overt clinical symptoms in the presence of myocardial siderosis.

Special attention is paid to the possibilities of modern therapy – chelator, allogeneic transplantation of hematopoietic stem cells, recommended for patients at an early age before the complications of iron overload have occurred. Having an HLA-identical donor remains the only definitive treatment for thalassemia. The newest therapeutic options are JAK2 inhibitors and recombinant proteins. They are becoming a promising alternative for improving hemoglobin levels and reducing frequent blood transfusions.

In the last part of the review, a special place is devoted to the study of new laboratory indicators for early cardiac dysfunction - microRNAs - them. It has been established that microRNAs are important both for the normal development of the cardiovascular system and for the occurrence of a number of pathological processes, such as cardiac hypertrophy, interstitial fibrosis and cardiac remodeling. The main ones related to the cardiovascular system are: MicroRNA-1, MicroRNA-21, MicroRNA-29, MicroRNA-30. It is assumed that they will have great potential in both diagnosis and targeted therapy in patients with BTM.

The author shows a very good and thorough knowledge of the subject of the dissertation.

The conclusions of the literature review are meaningfully formulated and logically argue the working hypothesis, the purpose and tasks of the dissertation work.

Purpose and tasks

The aim of the present dissertation is: "To find out whether there are early abnormalities in cardiac function in young BTM patients and whether they can be identified using some modern non-invasive echocardiographic indicators and cardiac damage-specific microRNAs".

To achieve the above-mentioned goal, 7 specific tasks have been correctly formulated.

Material and methods

The study is prospective, includes a total of 78 examined persons aged up to 25 years, of which 27 children and young patients with proven BTM, with an average age of 15.14 ± 5.83 years, who are undergoing treatment at the Expert Center for Coagulopathies and Rare anemias at UMBAL "St. Marina", as well as 51 healthy controls matched for sex and age. The study was launched after receiving permission from the Committee on Ethics for Scientific Research at the University of Medicine - Varna with Decision No. 84/27.06.2019 and covers the period July 2019 - June 2022.

The procedures performed were physical examination, ECG, laboratory parameters with blood count, serum ferritin, 6 microRNAs: microRNA-1, microRNA-21, microRNA-29, microRNA-30 and microRNA-150, selected on the basis of available scientific information on their involvement in cardiac pathology – acute cardiac injury, cardiac remodeling and fibrosis. Echocardiography was performed on an Esaote device, MyLabOmega model, manufactured in 2020 with a transducer in the range of 1-9 MHz depending on the age and physique of the subjects, following the recommendations of the American Association of Echocardiography in Childhood guarantees the reliability of the results obtained. It includes a detailed assessment of cardiac structures - left ventricular muscle mass, LV muscle mass indexed to body surface area (LVMi), calculation of LV volume, assessment of left ventricular systolic and diastolic function.

The applied modern statistical methods give grounds for the reliability of the obtained results.

Results and discussion

The section is presented on 47 pages. The results obtained by Dr. Kalina Ganeva end with a discussion in which they are thoroughly and competently compared with those of other authors.

Analysis of the obtained results showed significantly greater left ventricular muscle mass, indexed left ventricular muscle mass to body surface area (LVMi) and indexed LV volume to body surface area (LAVi). BTM patients had smaller body surface area, higher heart rate and lower blood pressure values compared to controls without the differences being significant. Children and young adults with BTM have preserved LV systolic function as assessed by ejection fraction. Nevertheless, it should be considered that GLS as an echocardiographic indicator identifies early heart disorders before the decrease in EF and the appearance of clinical symptoms, therefore the author recommends its use in routine clinical practice. Doppler measures of diastolic function in BTM patients showed that only the E/e' ratio in BTM patients was significantly increased compared to healthy controls. From the presented results, the dissertation student believes that not all criteria for assessing the diastolic function as impaired are met. The increased ratio of indexed left atrial volume to body surface area (LAVi) in more than half of the patients, as well as the tendency to rise in left ventricular end-diastolic pressure indicate initial changes in diastolic function with an increased risk of impairment in the future. No correlation was found between global longitudinal strain (GLS) and MRI T2* in patients with BTM, probably due to the small number of subjects.

Of the 5 types of microRNAs studied, associated with acute cardiac injury, cardiac fibrosis and cardiac remodeling, the values of microRNA-30 were significantly lower and those of microRNA-150 were significantly higher in BTM patients compared to controls, correlating with the increased LP and the available LKH..

Based on the obtained results and analyses, a protocol was prepared for the Echocardiography of patients with beta-thalassemia major.

Conclusions

The 9 conclusions made are clearly and logically formulated from the results and statistical analyzes in the context of the set goals and objectives.

Contributions

The 9 conclusions made are clearly and logically formulated from the results and statistical analyzes in the context of the set goals and objectives.

Contributions

The dissertation contributions, five in number, two of which are original and three of a confirmatory nature, are the result of research and well formulated.

The abstract in a volume of 80 pages reflects the main results and discussion, conclusions and scientific contributions of the dissertation work.

Publications

The author presents 4 publications related to the dissertation work (1 in print) in reputable Bulgarian medical publications referenced in an international database, meeting the requirements.

Recommendations

Given the high scientific value of the dissertation work and its relevance, I recommend the author to continue monitoring patients with BTM and collecting more data to optimize proactive behavior and adequate treatment of these children. To publish the obtained data in the foreign scientific periodical.

CONCLUSION

The dissertation presented to me by Dr. Kalina Binkova Ganeva contains data of an original and confirmatory nature and is a contribution to the development of pediatric science and, in particular, to pediatric cardiology and hematology.

For the first time in Bulgaria, new echocardiographic parameters related to myocardial deformation are used to detect early cardiac dysfunction in children and young patients with beta-thalassemia major, and a protocol for Echocardiography has been developed. For the first time in Bulgaria, specific microRNAs associated with heart damage in children and young patients with beta-thalassemia major are being investigated. The role of tissue Doppler in the early assessment of diastolic dysfunction in children and young patients has been confirmed. Global longitudinal strain (GLS) has been confirmed to contribute to the identification of early cardiac disorders with greater sensitivity than ejection fraction before the onset of clinical symptoms. The proposed protocol for echocardiographic examination in patients with beta-thalassemia major is particularly valuable for practice. In patients with BTM, changes in the expression of specific microRNAs were observed, which could be biomarkers of early cardiac damage.

The dissertation meets the requirements for awarding the educational and scientific degree "Doctor" laid down in the Law on the Development of the Academic Staff in the Republic of

Bulgaria and the Regulations for the Development of the Academic Staff of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

This gives me reason to vote with a positive vote and I strongly recommend to the respected members of the Scientific Jury to vote for awarding Dr. Kalina Binkova Ganeva the educational and scientific degree "Doctor" in the scientific specialty "Pediatrics", professional direction 7.1. Medicine, field of higher education 7. "Health care and sports".



28.04.2023

Prepared the opinion:

Assoc. Prof. Lachezar Radoslavov Marinov MD, PhD