Statement

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By order № P-109-187/15.03.2023 of the Rector of the Medical University of Varna, I`ve been assigned to set a statement as part of scientific jury for acquiring an educational and scientific degree – Doctor of Phylosophy (PhD) on the study with the title:

POSSIBILITIES OF SOME ECHOCARDIOGRAPHIC TECHNIQUES AND MICRORNAS FOR DETECTING SUBCLINICAL MYOCARDIAL DAMAGE IN CHILDREN AND YOUNG ADULTS WITH BETA-THALASSEMIA MAJOR

By PhD student Kalina Binkova Ganeva, MD

Educational organisation: Department of Pediatrics, Faculty of Medicine, Medical University – Varna
Field of Higher Education – 7.1. Medicine
Specialty: „Pediatrics"

CV data: Dr. Kalina Ganeva was born on 05.12.1986 in the city of Sliven. In 2006, she graduated from the High school of Mathematics "Dr. P. Beron" in the city of Varna, and in 2012 from the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. From 2013 to 2017 specializes in pediatric cardiology at the UMBAL "St. Marina", and for the period 2018-2020 - Pediatrics. She has two specialties - Pediatric Cardiology and Pediatrics. Since 2018, she has been an assistant-professor at the Department of Pediatrics, and since 2019, she has been a PhD student at the department.

Rlevence of the topic: Patients with beta-thalassemia belong to a group of diseases that are defined as rare, i.e. less than 5 per 10,000 population. For the medical professionals working with patients with rare diseases, it is a challenge to make a clinical decision on a case-by-case basis, given the scarce scientific information, the absence, usually, of large randomized clinical trials in the relevant field that provide clear and definitive guidelines for diagnostic and therapeutic actions and last but not least, the poor opportunity to gain personal clinical experience with a given rare disease. Beta-thalassemia is a disease that has seen tremendous success in patient life expectancy

over the past sixty years, from median survival into the second decade in the middle of the last century, to a life expectancy close to that of the general population for those born after 2000. And despite these great advances, in most patients, cardiovascular complications remain the main cause of morbidity and mortality. Most scientific publications studying various aspects of presentation and complication of patients with beta-thalassemia are serial observations or cover small cohorts of patients. Therefore, any scientific endeavor in the search for early biomarkers and imaging modalities of cardiovascular damage in patients with beta-thalassemia is important to enrich knowledge and a necessary step towards progress in preventing complications and disabilities in these patients.

Structure of the PhD work:

The study consists of 133 standard pages. The bibliography covers 147 sources, of which six are by Bulgarian authors, the rest are in English. The PhD work is illustrated with 14 tables, 39 figures and 2 appendices, which present the problem in a sufficiently clear, orderly and informative manner.

I believe that the structure of the scientific work meets the generally accepted requirements.

Aim of the study: The main objective of the scientific work is clearly defined: To determine whether there are early disturbances in cardiac function in young patients with beta-thalassemia and whether they can be identified by some modern non-invasive echocardiographic parameters and specific cardiac microRNAs.

In accordance with the main objective, seven tasks are defined - the assessment of physical development of children and young adults with beta-thalassemia, echocardiographic assessment of left ventricular function in patients with beta-thalassemia compared to healthy controls, investigation of a marker of iron overload – ferritin, study of the expression of specific microRNAs – microRNA-1, microRNA-21, microRNA-29, microRNA-30 and microRNA-150, as markers for IIF, remodeling and fibrosis in beta-thalassemia patients and healthy controls, to compare the results of the investigated microRNAs and the echocardiographic parameters of the patients and a correlation between the echocardiographic assessment of cardiac function and myocardial iron accumulation by means of the MRI T2* technique, which would allow to structure a protocol for echocardiographic examination and follow-up of cardiovascular status in patients with beta-thalassemia.

Review.

The literature review covers 29 pages, systematically and consistently, initially presenting the prevalence of the disease in Bulgaria and the world, the stages through which the treatment of patients passes over the years and the problems that are gradually overcome. An extremely thorough and comprehensive review of the scientific literature to date on cardiovascular complications in children and young adults with beta-thalassemia. Echocardiographic indices of systolic and diastolic function that change early in the course of the disease are discussed, as well

as the application of newer deformation techniques in this patient cohort. The inclusion of Bulgarian authors in the overview of the issue is also impressive.

Patients and methods:

The thesis includes a total of 78 examined subjects, of which 27 are children and young adults with confirmed beta-thalassemia, with an average age of 15.14 years, who are being treated at the Expert Center for Coagulopathies and Rare Anemias at University Hospital "St. Marina"-Varna, as well as 51 healthy controls matched for sex and age.

Given the nature of the disease, as a rare disease, the studied patient population is small in number. The methods of physical examination, the used laboratory methods and imaging studies are described in detail and clearly. Special attention is paid to the echocardiographic study, as well as to the study of non-coding small ribonucleic acids (microRNAs) - the two main studies underlying the scientific development. The statistical methods used give high reliability to the obtained results and are specialized for biostatistics.

Results and discussion:

The results are presented consecutively to the set goals and tasks, each of the obtained results is comprehensively discussed and illustrated.

1. Analysis of demographic, anthropometric and hemodynamic parameters of patients with beta-thalassemia major and comparison with the control group, showing that the case-control principle was followed and that patients with beta-thalassemia had a lower body surface area , but no significant difference was found regarding the hemodynamic parameters.
2. he analysis of the echocardiographic parameters of the patients and their comparison with those of the controls is extensive and thorough. It becomes apparent that even in children and young adults who are well transfused and chelated, changes in cardiac structure occur, the earliest of which are increases in left ventricular muscle mass and left atrial volume. This result is in agreement with previous scientific studies and confirms that the indicated data are significantly different from those of the controls. Regarding the ejection fraction and the global longitudinal strain, as expected, no differences were found with the control group. Special attention is paid to diastolic function, as it is considered that in the development of thalassemic cardiomyopathy, it precedes systolic disorders. Dr. Ganeva found a difference in systolic tissue velocity that increased in patients, albeit within reference values, compared to healthy controls. Another indicator showing a tendency towards future disturbance of diastolic function is the ratio of E/e', which is significantly increased in patients. Dr. Ganeva makes a thorough analysis and comparison of her results with the results of other authors who obtain the opposite of her data. The explanation for this discrepancy sounds logical and plausible - the time when the studies were conducted, the applied transfusion and chelation regimens, which differ from modern therapies, where patient participation is much greater than it was two or three decades ago.

3. The study of specific microRNAs associated with acute cardiac injury, cardiac fibrosis and cardiac remodeling is an innovative study in the dissertation work. Five types of microRNA were studied, and only two showed a significant difference with the controls - RQ has-miR-30a-5p and RQ has-miR-150-5p. Interpretation of the results is complex and challenging given the paucity of data to date in the scientific literature and lack of accurate reference values. Dr. Ganeva interpreted the results for obtained microRNA values in comparison with those available to date both for patients with beta-thalassemia and for groups of patients with cardiovascular involvement of other etiologies. Correlation analysis with some echocardiographic indicators revealed a moderate inverse relationship only between RQ has-miR-150-5p and indexed left atrial volume.

Conclusions:

There are nine conclusions in total and they correspond to the set goals of the scientific research. They are clearly defined and precisely formulated. The initial hypothesis, that in patients with beta-thalassemia the changes in cardiac function due to the disease and the deposition of iron in the myocardium can be observed from an early age and can be identified by means of modern echocardiographic methods including the tissue Doppler and the new deformation techniques is confirmed.

Weaknesses and shortcomings of the студь also critically discussed, the most important of which is the small number of examined patients.

The contributions of the PhD work that are of scientific and practical value are five in , three of them being original and two of a confirmatory characteristic. The use of deformation echocardiographic techniques in children with beta-thalassemia and the study of micro-RNA are original. This gives the research team full reason to recommend the routine use of deformation echocardiographic methods in the annual echocardiographic follow-up protocol of children with beta-thalassemia major, as well as to conduct an additional research on the role of cardiac specific micro-RNAs in this special patient cohort.

There are two publications, one in a refereed journal and two participations in national conferences, presenting the results of the thesis.

In conclusion, I can say that the dissertation work presented by Dr. Kalina Binkova Ganeva on the topic "Possibilities of some echocardiographic techniques and micro-scans for establishing subclinical myocardial damage in children and young adults with beta thalassemia major" fully meets the requirements of the law for the development of the academic staff in the Republic of Bulgaria and the regulations for its application at the MU-Varna, for the awarding of the educational and scientific degree "PhD", which is why I am convinced that I give a positive assessment.

Varna

17.04.2023

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