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Fund “Nauka” Project № 19007 Resume – Competition-Based Session 2019:

“Cardiac disorders in young patients with beta thalassemia major”

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Thalassemias are a group of hereditary diseases characterized by a decreased or absent synthesis of one or more of the globin chains and require lifelong blood transfusions. Complications due to iron deposition in internal organs occur with time. Chronic hemochromatosis affects the heart, the liver, and the endocrine glands. When proper treatment is conducted, the leading cardiac complications – iron overload cardiomyopathy and rhythm and conduction disorders are not clinically manifested in childhood. Nevertheless, the evidence showed myocardial iron overload begins in early childhood no matter conducting regular chelation therapy. According to literature data, up to 11.4% of children under 10 years of age already have myocardial iron overload. Bulgarian investigation of cardiac function in children with beta-thalassemia major (BTM), conducted in 2011, showed myocardial iron overload in up to 21.4%. That is why it is so important for early signs of myocardial damage to be detected before they are clinically manifested. This will ensure patients with better care and fulfilling life.

MicroRNAs (miRNA) are small, non-coding molecules of RNA which possess the potential to become biomarkers for several cardiac diseases. Studies on microRNAs are still in the field of scientific research, at this point, including in our own country.

The main hypothesis in the study is the presence of subclinical myocardial damage in asymptomatic young patients with beta-thalassemia major (BTM) as a result of secondary hemochromatosis. The purpose of the study was to detect whether there are signs of early myocardial damage, as well as the time of their appearance, by using novel echocardiographic techniques – tissue doppler, strain, and strain rate, as well as investigation of some specific microRNA. The main working methods were imaging (echocardiographic evaluation, magnetic resonance imaging (MRI) of the heart) and laboratory (iron overload markers and microRNAs).

The design of the study was prospective and included 50 subjects, 27 of whom were patients with BTM (13 girls and 14 boys) at an average age of 15.14 years (SD±5.83) and 23 healthy controls matched for sex and age. The study started in 2019 after receiving permission from the Research Ethics Committee at the Medical University of Varna with Decision No. 84, dated 27.06.2019, and covered the period of July 2019 – June 2022. The study was approved and funded by Fund “Nauka” with project № 19007.

The **results** of the specific microRNAs associated with cardiac damage were the following:

- MicroRNA-1 (RQ hsa-miR-1-3p) expression was detected in only 7 of the patients (25.9%), and 9 of the healthy controls;
- MicroRNA-21 (RQ hsa-miR-21-5p) and microRNA-29 (RQ hsa-miR-29b-3p) were detected in the serum of all of the subjects but without a significant difference ($p=0.3635$ and $p=0.4307$);
- MicroRNA-30 (RQ hsa-miR-30a-5p) was significantly downregulated in subjects in comparison to healthy controls ($p=0.0298$);
- MicroRNA-150 (RQ hsa-miR-150-5p) was significantly upregulated in the serum of patients compared to healthy subjects with $p<0.0001$.

According to the literature data, RQ has-miR-30a-5p, is found to be involved in cardiac fibrosis. Pathological cardiac remodeling is associated with decreased expression. The results of the study showed significantly lower expression of this specific microRNA in our patients in comparison to healthy subjects. When analyzing the currently available scientific data, we did not find a connection between hsa-miR-30a-5p and beta-thalassemia major which means that under normal circumstances, this specific miRNA is not found in the blood serum. The significant downregulation of RQ has-miR-30a-5p in the patients could predict future cardiac remodeling.

In our study, we found a significantly increased expression of RQ hsa-miR-150-5p in patients' serum compared to healthy controls. There is evidence that it is superior to NT-proBNP in predicting cardiac remodeling. However, unlike hsa-miR-30a-5p, hsa-miR-150-5p is considered disease-specific. It has several functions and is involved in different processes: it suppresses the alpha-globin chain expression and controls erythroid progenitor cells, and hsa-miR-150-5p expression decreases during erythropoiesis. Therefore, increased RQ hsa-miR-150-5p in the serum of the studied patients is more likely to be due to its function in erythropoiesis than to be a predictor for future cardiac complications.

The results in the present study of miRNAs in children and young patients with beta-thalassemia major present additional information about their role in the disease itself, but also in possible future cardiac complications. Given the potential role of miRNAs as biomarkers of cardiac damage, the altered expression of RQ hsa-miR-30a-5p and RQ has-miR-150-5p could predict future cardiac remodeling before clinical or imaging evidence for this occurs.

The scientific contributions of the present projects are as follows:

- For the first time in Bulgaria specific microRNAs associated with myocardial damage in young patients with beta-thalassemia major are investigated, and their diagnostic and prognostic role is evaluated.
- RQ has-miR-30a-5p could predict future cardiac remodeling in patients with beta-thalassemia major.

- Increased RQ hsa-miR-150-5p in the serum of the studied patients is more likely to be due to its function in erythropoiesis, as well as, to be a predictor for future cardiac complications.
- As part of the project, the cardiac function of children and young adults with beta-thalassemia major was evaluated using novel techniques for myocardial deformation – strain and strain rate. Up until now, such research has not been carried out in Bulgaria. Literature data for this population regarding these specific techniques even outside the country are scarce.
- The diagnostic value of tissue Doppler, strain, and strain rate echocardiographic methods as a predictor of myocardial damage in young patients with beta-thalassemia major was assessed.
- An algorithm will be created for the application of innovative, non-invasive echocardiographic methods for diagnosis and following-up of cardiovascular status in patients with beta-thalassemia major from an early age.