Review

I. General presentation of the procedure and Ph.D student

The presented set of materials on paper and electronic media is in accordance with:

- Order № P-102-187/15.03.2023
- law on the development of the academic staff of the Republic of Bulgaria
- Ph.D work
- Abstract
- Publications
- Set of documents of the applicant

II. Short biography of the Ph.D student

She graduated from "Dr. Petar Beron" high school in 2006, and later on in 2012 she graduated from Medical University, Varna. Then in 2012 she started working as a doctor at the Clinic of Paediatrics, "Sveta Marina". On the 1st of July 2013 she started her residency in pediatric cardiology at the Medical University "Prof. Dr. Paraskev Stoyanov", city of Varna. In September 2017 was appointed as a part-time assistant professor at the Department of Pediatrics - Medical University, Varna. In the month of January 2018 acquired a specialty in pediatric cardiology. In October 2018 after a successful competition, she started working as a full-time assistant in the Department of Pediatrics, University of Varna. From 2019 dr Ganeva is a full-time doctoral student at the Department; in 2021 she acquired the specialty of Pediatrics.

In addition, she is a member of the Bulgarian Pediatric Association, Bulgarian Medical Association, the European Society of Cardiology, a member of the European Association of Pediatric Cardiologists. Moreover, she is fluent in English and is able to work excellently with computers - Windows MS Office.

III. Relevance of the topic and appropriateness of the set goals and tasks

The dissertation is devoted to the evolution in the clinical course, the expected complications of the cardiovascular system and the possibility of the diagnosis of myocardial hemosiderosis in subclinical myocardial manifestation, using all imaging methods to evaluate these complications in children and young adults with homozygous Beta thalassemia.

Although the knowledge of homozygous Beta thalassemia is now considered to be complete, cardiac complications are still a serious challenge, concerning both life expectancy and quality of life. According to published data, the frequency of Beta thalassemia in our

country is 3.66 per 100,000 people, with 92.22% of patients having the severe, major form of the disease, and the rest - thalassemia intermedia.

Regular hemotransfusions and the subsequent natural hemolysis of donor erythrocytes lead to an increase in iron levels in the blood, and the human body does not have a mechanism by which to excrete it. In the 1960s, Deferoxamine was first introduced as the first iron overload chelator. Deferiprone is the second chelator, orally taken, and the third confirmed drug is Deferasirox - now much more convenient for intake-once.

The application of chelators leads to a significant improvement in the cardiac status of patients, but does not definitively solve the problem of damage to the cardiovascular system.

Despite the undeniable progress of medicine over the past forty years, thanks to which the survival of patients has dramatically improved, cardiovascular damage remains the leading cause of morbidity and mortality in up to 71%. Heart failure and arrhythmias occur in 9% and 10%, respectively, and about 13% of them develop pulmonary hypertension. At the onset of cardiac dysfunction, patients are asymptomatic and the clinical manifestation of cardiac damage is the end phase of thalassemic cardiomyopathy.

Therefore, the dissertation student sets herself the goal of establishing the early disorders in cardiac function, to be identified by means of some modern non-invasive echocardiographic indicators and microRNAs specific for cardiac damage.

All this determines the high degree of scientific and scientifically applied relevance of the developed dissertation work.

IV. Knowing the problem

The literature review was written on 147 literary sources, most of them contemporary and published recently. In the presented introduction, a retrospective analysis of the data on thalassemic syndromes in a global and national aspect is made.

The literature review is thorough and analytical, and the long road to find the diagnostic and therapeutic optimum to ensure a long and good quality life for these patients is well exposed. Of course, the emphasis of the study is on myocardial hemosiderosis, as the main cause of fatal outcome of the disease.

V. Methods

The doctoral student knows all non-invasive echocardiographic methods for the early diagnosis and prevention of the clinical symptoms of thalassemic cardiomyopathy extremely well. This is quite real, because he is a specialist in pediatric cardiology. The advantages and disadvantages of the echocardiographic studies performed so far are discussed:

- Telediastole diameter of left ventricule

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- Ejection fraction of left ventricule
- Fraction of shortness
- Width of intraventricular septum and back wall of left ventricule
- Left ventricule muscle mass
- Telesistole diameter of left atrium
- Indexed volume of left ventricule vs body surface

An evaluation of myocardial deformation was made by trekking estimations of global longitudinal strain.

An assessment of left ventricular diastolic function was performed personally by the PhD student, and tissue Doppler and GLS feasibility analysis was performed. All of these labor-intensive and demanding echocardiographic skills are aimed at evaluating early cardiac dysfunction in asymptomatic children and young adults with homozygous beta thalassemia.

Of course, the so-called gold standard for assessment of iron burden with MRIT_2* . An assessment of the ferritin level was also made against the background of the chelation treatment being carried out.

27 patients were included in the scientific study, of which 13 were girls and 14 were boys. The mean age of the subjects was 15.14 years (SD+/- 5.83), as well as 51 controls (healthy individuals aged 0-25 years). The analysis of the obtained results, namely: higher heart rate, lower arterial pressure, significantly greater left ventricular mass, increased indexed left atrial volume, preserved left ventricular systolic function and only statistically significant with higher values ratio E/ e (lack of correlation between GLS and MRI2*) is the reason for Dr. Ganeva to look for another more sensitive research method, as a predictor of the early symptoms of thalassemic cardiomyopathy.

According to this aim, the scientific research focuses on the study of microRNAs as new laboratory indicators of early cardiac dysfunction. They are small non-coding RNA molecules and currently more than 3000 different species have been recorded in the human genome.

In cardiac muscle, microRNA-1, microRNA-133, microRNA-499 and microRNA-208 are represented. The significance of microRNA-29, microRNA-30 and microRNA-150 as markers of heart failure, remodeling and fibrosis in patients with Beta thalassemia and healthy controls was analyzed. The dissertant reports encouraging results in the expression of microRNA-30. The amount was significantly lower compared to healthy controls (1.0148+/-

0.66 vs 1.55563+/- 0.99). The most convincing results were obtained when evaluating the expression of microRNA-150. In these cases, the mean values of all patients were 42.0431+/- 30.032, significantly higher than healthy controls 13,7688+/- 24.049.

An attempt was also made to look for a correlation between some echocardiographic parameters and certain microRNAs. Dr. Ganeva found a moderate inverse relationship only between Has-miRNA-150-6p and LAVi, as well as between Has-miRNA-150-5p and increased LAVi. A weak direct relationship was established between RO Has-miRNA-30a-5p and left ventricular hypertrophy.

VI. Characteristics and evaluation of the dissertation

The dissertation is written on 130 pages, illustrated with 14 tables, 19 figures and 2 appendices. The figures are of good quality and sufficiently informatively demonstrate the established statistical dependencies.

The overview is specified correctly and according to the requirements for a dissertation. The volume distribution of the presented material allows an adequate presentation of the information. It is distributed proportionally in separate subsections.

Dr. Ganeva aims to evaluate the possibilities of some echocardiographic techniques and microRNAi for establishing subclinical myocardial damage in children and young adults with Beta thalassemia major.

To achieve her goals, she sets seven tasks. The tasks are clearly and accurately formulated and help to achieve the set goal.

A discussion of the result follows the conclusions. In it, Dr. Ganeva shows in-depth reasoning and conclusions of the obtained results. The comparison of own results with those of other studies concerns all parameters of imaging studies. At the same time, the shortcomings of some of the methods used are critically commented on.

The dissertation concludes with nine conclusions that reflect the most essential part of the result. The conclusions are in accordance with the tasks set and follow the obtained results and the discussion. They are clear, precise and well worded.

VII. Contributions and significance of the development for science and practice

Dr. Ganeva offers: an original contribution concerning the study of specific mRNAs associated with cardiac damage in children and young patients with Beta thalassemia. There are three confirmatory contributions of practical significance and applicability.

VIII. Abstract

The submitted abstract is written in 79 pages and is structured in the same way as the dissertation. After a short introduction that points to the purpose of the scientific research, the aims and objectives are stated.

The material and methods are presented in 11 pages and outline the wide range of research carried out by the PhD student.

The own results and discussion are summarized in 36 pages, illustrated with 36 figures and 8 well-made tables, demonstrating the most significant results. The summary is short, but the parameters of the developed problem and the achieved results are clearly outlined. The conclusions drawn (9) are specific and confirm the scientific-applied nature of the developed dissertation work.

IX. Recommendations for future use of dissertation contributions

Dr. Ganeva complied with all the recommendations and critical notes from the internal protection. I recommend in its further development to implement the studied data in clinical activity and conduct a comparative study between two independent trained researchers working on the problem of prevention, clinical approach and therapeutic behavior in children and young adults with secondary cardiac hemosiderosis with homozygous Beta thalassemia to determine the comparability of results between investigators.

X. Conclusion

The dissertation contains scientific, scientific-applied results that represent a contribution to science and meet the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria (ZRASRB). The presented materials and dissertation results fully comply with the requirements of the MU-Varna.

Dr. Ganeva possesses good theoretical knowledge and serious professional skills in the scientific specialty of Pediatrics and Children's Cardiology, demonstrating qualities and skills for independent conduct of scientific research.

But I would express my astonishment at the fact that the dissertationer has not noted anywhere that the beginning of knowledge in all aspects of Beta thalassemia in Bulgaria is associated with the name of Prof. G. Petkov, long-time head of the Department of Pediatrics at the Medical Faculty, Trakia University, Stara Zagora.

Back in 1986, he defended a spectacular doctorate - Studies on thalassemic syndromes in childhood (287 pages and a bibliographic reference of 520 authors). In 2003 the monograph Thalassemic Syndromes is published under his supervision. Together with a team, publications in prestigious international journals followed (Hemoglobin, Acta Haematologica, Journal of Biochemical and Biophysical Methods and a number of others). The publications

are related to clarifying the genetic profile of these patients, haplotyping and some new unstable hemoglobins as a cause of hemolysis. Very early in the periodical, a scientific team led by Prof. Petkov published the echocardiographic changes in patients with Beta thalassemia (1988, Pediatrics magazine).

After determining the varieties of the different types of mutations and their relationship with the phenotypic appearance in these patients (dissertation Assoc. Chakarova, 1991), an answer was found to the question of the different time interval of hemotransfusions in these patients.

I would like to draw Dr. Ganeva's attention to the fact that innovative research must also be consistent with the existing foundation of the problem - cardiac hemosiderosis in patients with Beta thalassemia.

A number of our studies have shown that complications can be localized in the excretory system and in the eyes. Therefore, efforts in the prevention of early complications should also be aimed at the quality of life.

The number of examined patients is relatively small, but therefore I assume that the significance of the examined mRNAs is at the hypothetical stage. I also accept the fact that these patients have serious mental deprivation and are not particularly inclined to cooperate in the research. But since the scientific study promises new possibilities for reducing the complications of excess iron load on the cardiovascular system, the results and conclusions obtained, although in their infancy, promise undeniable benefit in the future.

However, analyzing the conclusions drawn, more work should be done in the direction of early diagnosis in proving the risk of giving birth to children with homozygous Beta thalassemia, because despite the other treatment options, apart from the conventional one (stem cell transplantation, gene therapy) there are more what to wish for.

Due to what I stated above, I give my positive assessment to the research conducted, presented by the above-reviewed dissertation work, abstract, the achieved results and contributions and I propose to the honorable jury to award the educational and scientific degree "Doctor" to Dr. Kalina Binkova Ganeva in a doctoral program in Pediatrics.

26th of June, 2023
Stara Zagora

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Prof. Dr. P. Chakarova, PhD