ABSTRACT

of the dissertation for the Doctor of Philosophy degree (PhD) educational program «8D05102-Biomedicine»

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«Study of the role of plasmatic microRNAs in type 2 diabetes mellitus inflammation and vascular system state related»

General description of the research. This dissertation work is dedicated to a comprehensive investigation of plasma microRNA expression closely associated with inflammatory processes and vascular dysfunction in type 2 diabetes mellitus. The study places particular emphasis on the role of microRNAs in endothelial dysfunction, the enhancement of oxidative stress, and the impact of these factors on the pathogenesis of vascular complications. In addition, the research evaluates the biomarker and prognostic significance of microRNAs, as well as their potential for the early diagnosis and prevention of diabetes-related macrovascular and microvascular complications.

Significance of the research. Type 2 diabetes mellitus is one of the most common chronic diseases, characterized by the development of cardiovascular complications. Macrovacular disorders, such as coronary artery disease, stroke, and peripheral arterial disease, significantly increase mortality risk among patients with type 2 diabetes. Current diagnostic methods do not allow for the early identification of vascular complication risks, which contributes to disease progression and a decline in patients' quality of life. Therefore, investigating the mechanisms underlying vascular complication development in type 2 diabetes and identifying novel biomarkers represents a pressing scientific and clinical challenge.

In recent years, particular attention has been given to microRNA molecules, which play a crucial role in regulating gene expression at both the intracellular and extracellular levels. Studies have demonstrated the association of microRNAs with inflammatory processes, endothelial dysfunction, and oxidative stress, confirming their pivotal role in the pathogenesis of cardiovascular diseases. In particular, key signaling pathways such as NF-κB and the NLRP3 inflammasome are regulated by microRNAs, influencing the amplification or attenuation of inflammatory responses.

Oxidative stress plays a major role in the development of type 2 diabetes. An imbalance in cellular antioxidant systems enhances lipid peroxidation and leads to reduced activity of superoxide dismutase and catalase, contributing to vascular wall damage and the progression of endothelial dysfunction. Moreover, in type 2 diabetes, inflammatory mediator levels remain persistently elevated, indicating a chronic inflammatory process and its detrimental effects on the cardiovascular system.

The findings from such studies may serve as a basis for developing new diagnostic and prognostic methods aimed at more accurately assessing the risk of cardiovascular complications in patients with type 2 diabetes mellitus.

The purpose of the research. Identification of new prognostic criteria based on the study of the relationship between plasma microRNA expression, oxidative stress markers, and inflammatory markers in the development of macrovascular complications in type 2 diabetes mellitus.

To achieve the goal, the following six tasks were set:

1. To determine the relative expression levels of key microRNAs (hsa-miR-21-5p, hsa-miR-126-3p, hsa-miR-146a-3p, hsa-miR-155-5p, hsa-miR-210-3p, hsa-miR-484-5p, and hsa-miR-27a-3p), as well as biochemical parameters, in patients with type 2 diabetes mellitus (with and without vascular complications) and in a control group;
2. To investigate differences in the activity levels of oxidative stress markers (LPO, AOPP, NOx) and antioxidant enzymes (SOD, CAT, GPx, GRd, G6PD, GSSG/GSH ratio) associated with the presence of vascular complications;
3. To assess the levels of pro-inflammatory and anti-inflammatory cytokines (IL-6, IL-8, IL-10, IL-18, MCP-1, TNF-α) in the plasma of patients with type 2 diabetes mellitus and determine their association with the development of vascular complications;
4. To identify microRNAs showing the highest correlation with inflammation and oxidative stress markers and evaluate their diagnostic significance;
5. To determine the influence of sex, lipid profile, and other clinical factors on microRNA expression levels and inflammatory marker content in patients with type 2 diabetes mellitus.

The research objects and materials. Peripheral blood samples from patients with type 2 diabetes mellitus (with and without vascular complications), as well as blood samples from healthy individuals included in the control group, were used as the study objects.

Research Methods. In this study, biochemical methods were used to determine the levels of glucose, creatinine, triglycerides, cholesterol (total, LDL, HDL), insulin, and HbA1c. During the course of the dissertation work, biomaterial samples were collected, anthropometric parameters were measured, and lipid profile analysis was performed (enzymatic methods), along with fluorescence spectroscopy (Bio-Tek Instruments Inc., Winooski, VT, USA), spectrophotometry, UV spectrophotometry, enzyme-linked immunosorbent assay (ELISA), nucleic acid extraction, reverse transcription PCR, and quantitative real-time PCR.

Statistical analysis was carried out using IBM SPSS Statistics for MacOS, version 20.0, and GraphPad Prism; methods applied included testing for normality of distribution, analysis of variance, correlation and logistic regression analysis, as well as ROC curve construction.

The scientific novelty of the research:

The most significant scientific results of the dissertation are:

For the first time, a comprehensive study was conducted on the specific features of plasma microRNA expression associated with the development of macrovascular complications in patients with type 2 diabetes mellitus. It was established that oxidative stress and chronic inflammatory processes, which reduce the activity of

antioxidant system enzymes and lead to the accumulation of oxidative damage products, influence microRNA levels.

MicroRNAs closely related to inflammatory markers and oxidative stress indicators were identified, and their functional role in the pathogenesis of vascular complications was substantiated. For the first time, a comprehensive model for predicting the risk of developing diabetic angiopathies based on the integration of molecular and biochemical parameters was proposed. The data obtained in the study confirmed the clinical significance of microRNAs as diagnostic and prognostic biomarkers and outlined the prospects for their implementation in the system of early detection and prevention of vascular complications in type 2 diabetes mellitus.

Theoretical significance of the research:

MicroRNAs play an important role in regulating oxidative stress and endothelial dysfunction; therefore, considering them as markers for assessing the progression of type 2 diabetes mellitus makes it possible to ensure personalized patient monitoring and to choose optimal treatment strategies for the prevention of cardiovascular complications.

The results obtained expand our understanding of the molecular mechanisms underlying the pathogenesis of vascular complications. Circulating microRNAs and oxidative stress markers are involved in the development of endothelial dysfunction in hyperglycemia and in the pathophysiology of type 2 diabetes mellitus. They can serve as predictors of vascular damage and cardiovascular complications. These markers enable prediction of disease progression even before cardiovascular complications appear, which may help prevent vascular injury and reduce the risk of cardiovascular mortality among diabetic patients.

Epigenetic studies confirm the influence of environmental factors on the regulation of metabolic processes. In this context, microRNAs have the potential to serve as physiopathological biomarkers capable of reflecting disease dynamics and the effectiveness of ongoing therapy. The present study confirms the clinical significance of molecular biomarkers and provides a theoretical foundation for the further development of personalized monitoring methods for patients with type 2 diabetes mellitus.

Practical significance of the research:

The results of this study can be used for the early identification of patient groups with type 2 diabetes mellitus who are at high risk of developing macrovascular complications. Changes in the expression of microRNAs associated with endothelial protective mechanisms and inflammatory processes may serve as a basis for developing new therapeutic approaches aimed at regulating vascular function. The prognostic model, which includes molecular and biochemical markers, will help more accurately assess the risk of vascular complications and contribute to the improvement of preventive strategies in clinical practice.

The results of the proposed research work were implemented in the lecture and seminar courses for the first-year doctoral program in “8D05102 - Biomedicine” within the discipline “Regulation of Metabolism and Energy,” as well as for the second-year master’s program in “7M05102 - Biomedicine” within the discipline

“Applied Endocrinology” at the Department of Biophysics, Biomedicine, and Neuroscience, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University.

The main provisions for the defense:

- The expression levels of microRNAs miR-21-5p, miR-126a-3p, miR-146a-3p, miR-155-5p, miR-210-3p, miR-484-5p and miR-27a-3p were studied in patients with type 2 diabetes mellitus, and their association with the development of macrovascular complications was analyzed;
- Differences in the activity levels of oxidative stress markers (LPO, AOPP, NOx) and antioxidant enzymes (SOD, CAT, GPx, GRd, G6PD, and the GSSG-GSH ratio) were identified between patients with type 2 diabetes without complications and those with vascular complications;
- A relationship was established between inflammatory dysregulation and changes in microRNA expression, and their role in the development of vascular complications was confirmed;
- Key microRNAs that show strong correlation with markers of inflammation and oxidative stress were identified, confirming their pathogenic significance;
- A prognostic model was developed based on molecular and biochemical indicators, enabling the assessment of the risk of vascular complications.

Personal contribution of the dissertation student to the complication of the results of scientific work proposed for defense. The results of this dissertation were obtained with the direct involvement of the author. The researcher contributed to the development of the study concept, the formulation of its aims and objectives, and the planning of the experiments. The author participated in conducting the research, processing and analyzing the obtained data, as well as in the preparation of scientific publications.

The levels of research organization. The research described in this dissertation was performed on the physiological, biochemical and epigenetic level.

Connection of work with scientific research program. The dissertation was carried out within the framework of scientific programs aimed at studying the pathogenesis of vascular complications in type 2 diabetes mellitus at the molecular and biochemical levels. The research focused on the influence of oxidative stress, inflammatory processes, and impairments in the antioxidant defense system on microRNA expression, as well as their role in the development of endothelial dysfunction and macrovascular complications. The study aligns with scientific objectives directed toward assessing the biomarker and prognostic value of microRNAs and developing a diagnostic model for predicting the risk of vascular complications based on these markers.

The dissertation was conducted within the framework of an international project (Grant for the Research Project by the Fundación Eugenio Rodríguez Pascual, Call 2021, CIBERfes (CB16-10-00238, ISCIII)) in the Molecular Biology Laboratory at the Center for Biomedical Research of the University of Granada, under the supervision of Professor, PhD Iryna Rusanova. Part of the research was also carried out at the “Biophysics, Biomedicine, and Chronobiology” laboratory of the

Department of Biophysics, Biomedicine, and Neuroscience, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, under the academic supervision of the national scientific advisor, Professor N.T. Ablaikhanova.

Approbation of the work. The main findings and results of the dissertation were presented at the following international scientific conferences and symposia:

- International Scientific Conference of Students and Young Researchers "Farabi Álemi" (2021–2024, Almaty, Kazakhstan);
- EUROMIT 2023 (Bologna, Italy);
- BALS-2024 Workshop (Granada, Spain);
- BIO Web of Conferences 100, 01008 (2024).

Publications. The majority of this dissertation content was published in 14 scientific works, including 2 articles in foreign journals Antioxidants (IF 7.675, Q1), International Journal of Molecular Sciences (IF 6.2, Q1) included in Scopus and Web of science databases; 4 articles in scientific journals recommended by Committee for the Provision of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan (CCESF MES RK); 8 theses were published in collections of materials of foreign and international-republican conferences.

Dissertation structure. This dissertation is written in 130 pages, and contains notations and abbreviations, introduction, literature review, materials and methods, results and discussions, conclusions, references and appendices from 226. Contains 11 tables, and 31 figures.