

ABSTRACT

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

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«Study of microRNA expression and Oxidative Stress Status as biomarkers of Cardiovascular Complications in patients with Type 2 Diabetes Mellitus»

General description of the research. The dissertation is dedicated to a comprehensive study of microRNA expression, oxidative stress status and inflammatory process and their role as biomarkers of cardiovascular complications in patients with Type 2 Diabetes Mellitus.

Significance of the research.

Type 2 diabetes mellitus is a complex metabolic disease prevalent worldwide, characterized by hyperglycemia resulting from tissue resistance to insulin. Patients often remain asymptomatic despite elevated blood glucose levels, complicating diagnosis. The clinical parameters used for diagnosis lack specificity, necessitating the identification of circulating markers for disease diagnosis and prognosis. Complications of type 2 diabetes include damage and dysfunction of vital organs such as the eyes (retinopathy), kidneys (nephropathies), nerves (neuropathies), and coronary vessels (cardiovascular disease).

The pathogenesis of T2DM is multifaceted, involving various pathophysiological and chronic inflammatory processes, including oxidative stress. Changes in the immune status of patients associated with impaired glucose metabolism can lead to vascular endothelial dysfunction. MicroRNA expression influences redox status and inflammatory processes, serving as biomarkers for cardiovascular complications. While numerous studies have investigated microRNA expression in diabetes, its role in vascular complications warrants further investigation.

In recent years, microRNAs (miRNAs), small non-coding RNAs approximately 18-22 nucleotides in length, have been extensively studied as crucial regulators of cellular functions. MiRNAs participate in various pathological processes and have been detected and extracted from various biological fluids, including whole blood, serum, and plasma, they are very stable molecules and are easily detected in the bloodstream. Circulating miRNAs exhibit altered expression patterns depending on the physiological state, aiding in the prediction, diagnosis, and monitoring of metabolic diseases, including diabetes. The identification and utilization of biomarkers involved in disease progression enable more accurate determination of exacerbation processes and facilitate the development of preventive strategies against vascular complications in T2DM.

It has now been established that oxidative stress is a component of metabolic disorders in diabetes mellitus and plays a key role in the development of macro- and microangiopathies in T2DM.

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Increased oxidative metabolism in the cell contributes to the highest electron escape in the electron transport chain, increased redox potential, and generation of the superoxide radical ($O_2^{\bullet-}$).

Numerous studies have convincingly demonstrated that chronic tissue inflammation is a key contributor to the development of T2DM. Inflammation serves as the initial response of the immune system to tissue damage and is integral to the pathogenesis of various vascular diseases such as atherosclerosis and diabetic angiopathy. Consequently, cytokines, hormone-like protein substances produced by activated immune cells involved in intercellular communication, hematopoiesis, and inflammation, lack specificity for antigens and mediate immune responses and intersystem interactions.

Given the foregoing, a comprehensive and systematic approach based on the study of miRNA expression, redox status, and inflammatory processes as biomarkers of cardiovascular complications in patients with type 2 diabetes mellitus hold significant promise.

In connection with the foregoing, a comprehensive and systematic approach based on the study of miRNA expression, redox status, and inflammatory processes as biomarkers of cardiovascular complications in patients with type 2 diabetes mellitus is very promising.

The purpose of the research. Study of microRNA expression, oxidative stress, and inflammation as new diagnostic markers for cardiovascular complications in patients with type 2 diabetes mellitus.

To achieve the goal, the following tasks were set:

1. Comparative analysis of biochemical parameters (HbA1c, glucose, insulin, HOMA-IR index, total cholesterol, triglycerides) in the blood of patients with type 2 diabetes mellitus.

2. To identify the relative expression levels of hsa-miR-21-5p and hsa-miR-126-5p in the blood plasma of patients with type 2 diabetes mellitus.

3. To assess the redox status by analyzing markers of oxidative damage in the blood of patients and indicators of the endogenous antioxidant protection system.

4. To determine the spectrum of inflammatory markers (IL-6, IL-10, IL-18, and TNF- α) in the plasma of patients with type 2 diabetes mellitus and to analyze the correlation between inflammation markers, redox status, and relative microRNA expression levels.

5. To study the diagnostic value of markers associated with the presence of type 2 diabetes mellitus and its vascular complications in the study groups.

The research objects and materials. Peripheral blood samples from controls and DM2T patients with and without vascular complications.

Research Methods.

Anthropometric methods, lipid profile (enzymatic methods), the method of fluorescence spectroscopy (Bio-Tek Instruments Inc., Winooski, VT, USA),

spectrophotometry method, UV-spectrophotometry method, enzyme-linked immunosorbent assay (ELISA), nucleic acid extraction, reverse transcription PCR, and real-time PCR methods. Statistical analysis was carried out by IBM SPSS Statistics for MacOS, version 20.0, graphs were built using version 6 of GraphPad Prism.

The scientific novelty of the research.

The most significant scientific results of the dissertation are as follows:

- for the first time, multimap studies were conducted on biomaterial obtained from patients in a Mexican population diagnosed with type 2 diabetes mellitus, both without cardiovascular complications and with complications;
- circulating blood biochemical parameters (HbA1c, glucose, insulin, HOMA-IR index, total cholesterol, triglycerides) were investigated in patients with type 2 diabetes mellitus, both without and with vascular complications;
- the relative expression level of free circulating hsa-miR-21-5p, hsa-miR-126-5p as biomarkers at the epigenetic level was revealed;
- identified the redox status associated with oxidative stress in patients with type 2 diabetes mellitus, both without and with vascular complications;
- the concentration of inflammatory cytokines (IL-6, IL-18, IL-10, TNF- α) in the blood plasma of the studied groups of patients was determined;

The findings demonstrate the diagnostic value of certain markers associated with the development of type 2 diabetes mellitus and its vascular complications in the studied groups.

Theoretical significance of the research.

The results of this study make a significant contribution to understanding the epigenetic mechanisms of type 2 diabetes, circulating miR-21 and miR-126, as well as biomarkers of inflammation and oxidative stress. Changes in microRNA expression play an important role in diagnosing complications of diabetes mellitus and in the development of micro- and macrovascular diseases.

Patients with diabetes engage in self-monitoring of blood glucose levels to reduce the risk of complications such as diabetic retinopathy, neuropathy, and atherosclerosis. However, despite achieving good glycemic control, some patients still develop vascular complications. The mechanisms underlying metabolic memory are not fully understood, but it is hypothesized that elevated glucose levels may induce damage to cells surrounding blood vessels, further impairing their function. This phenomenon can result in a deterioration of microcirculation, an elevated risk of thrombosis, and the formation of blood clots, ultimately contributing to the development of vascular complications.

Modern molecular biomarkers used in clinical practice for the diagnosis of diabetes do not have sufficient sensitivity to predict, emergence and progress of complicated diseases. The study of epigenetic mechanisms, in particular, the expression of miRNAs, contributes to the expansion of the boundaries of our view, provides an opportunity to develop new methods of diagnostics and personalized means of treating patients. MicroRNA expression plays an important role on the development of oxidative stress and chronic inflammation associated with the

manifestation of diabetic complications. These new data are an important step in understanding the molecular mechanisms underlying the development of type 2 diabetes and its complications and may provide the basis for developing effective ways to diagnose and prevent the disease.

Practical significance of the research.

The investigation of enzymatic and non-enzymatic antioxidant system factors allows a deeper comprehension of their impact on the efficacy of the body's antioxidant defense against the development of vascular endothelial dysfunction. These findings will contribute to the effectiveness of preventive medicine in addressing type 2 diabetes mellitus development, as well as the formulation of optimal protocols for the management and treatment of patients at risk of complications.

The results of the proposed research work were implemented in lectures, and practical classes in the disciplines "Molecular endocrinology", and "Molecular membranology" for the curriculum of the educational program «7M05102-Biomedicine» of 2nd course of the Department of Biophysics, Biomedicine and Neuroscience, Faculty of Biology and Biotechnology, Kazakh National University named after Al-Farabi. Act of introduction of completed research work into the educational process No.3 2023 – Application A.

The main provisions for the defense:

1. A difference was found in the comparative analysis of biochemical parameters (HbA1c, glucose, HOMA-IR index) in the blood of the control group, patients with T2DM without complications and with complications.

2. The relative level of microRNA expression as a prognostic biomarker in patients with T2DM without complications and with complications was analyzed.

3. It was revealed that the redox status of the control group differs from that of patients with T2DM without vascular complications and with complications.

4. It was found that the indicators of IL-6, IL-18, IL-10, TNF- α associated with inflammation in blood plasma differ in the control group, in patients with T2DM without complications and with complications, and the correlation of the results was analyzed.

5. It was proposed to consider hsa-miR-21-5p, SOD, LPO, IL-6 as prognostic markers associated with the pathogenesis of T2DM and the occurrence of vascular complications.

Personal contribution of the dissertation student to the complication of the results of scientific work proposed for defense. All the results of the dissertation work were obtained in the presence and personal participation of the author. The dissertator independently analyzed the literature data on the topic of the study, experiments, processed and analyzed the results of the study, and wrote and designed the manuscript of the dissertation.

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

Approbation of work. The main provisions of the dissertation and research results were reported and discussed at international scientific conferences, “Kazakh

National Medical University named after S.D.Asfendiyarov” NJSC, RSE “Institute of Genetics and Physiology” of the Ministry of Education and Science of the Republic of Kazakhstan, NJSC “Kazakh National Women's Teacher Training University”.

Connection of work with the scientific research program.

The work was carried out within the framework of the international project of the Laboratory of «Comunicacion Intercellular» Grupo de Investigacion CTS-101 of the Center for Biomedical Research of the University of Granada (project leader - PhD, Professor Rusanova Iryna).

Publications. The majority of this dissertation content was published in 20 scientific works, including 4 articles in foreign journals (Antioxidants IF-7.675, Q1., Oxidative Medicine and Cellular Longevity, IF-6.543, Q2, Journal of Pharmacy and Nutrition Sciences Q3) including in Scopus and Web of Science databases; 3 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); 13 theses were published in collections of materials of foreign and international-republican conferences.

Dissertation structure. This dissertation is written in 131 pages and contains notations and abbreviations, an introduction, a literature review, materials and methods, results and discussions, conclusions, references, and appendices from 236. Contains 11 tables, and 29 figures.