«KARAGANDA MEDICAL UNIVERSITY»

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**ABSTRACT**

of dissertation for obtaining scientific degree of Philosophy Doctor: «Glycemic regulation biomarkers in patients with type 2 diabetes mellitus and cardiovascular events»

Specialty: 6D110100 «Medicine»

Author: PhD student Sheryazdanova Dinara Nurlanovna

Scientific adviser:

Laryushina Yelena Mikhailovna, Candidate of Medical Sciences, Рrofessor, Head of Internal Medicine Department of «Karaganda Medical University» NCJSC

Scientific adviser:

Муравлёва Лариса Евгеньевна Doctor of Biological Sciences, Professor of Biological Chemistry Department, «Karaganda Medical University» NCJSC

Scientific adviser:

Vaiva Hendrixson, MD, РhD, professor of Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

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**Relevance of the study**

According to the World Health Organization, diabetes mellitus (DM) is recognized as one of the significant causes of morbidity and mortality from non-communicable diseases worldwide [1]. The incidence of diabetes in 2021, as reported by the World Diabetes Federation, is 537 million adults aged 20–79 years; every tenth person in the world lives with diabetes. Additionally, another 541 million people are at high risk of type 2 diabetes due to impaired carbohydrate metabolism [2]. The treatment of patients with diabetes imposes a substantial burden on the budgets of national healthcare systems. Each country spends at least <5% annually on diabetes treatment [3].

Typically, the onset of diabetes is preceded by a prolonged prediabetic phase. Furthermore, prediabetes, along with type 2 diabetes, is considered a pathological condition that serves as an independent predictor of adverse cardiovascular outcomes [4]. Among the most common complications associated with prediabetes are myocardial infarction, stroke, chronic kidney failure, diabetic foot with the development of gangrene, loss of vision, and neuropathy. Diabetes poses an extreme danger in the case of untimely diagnosis, which, in turn, carries the risk of developing macrovascular and microvascular complications and may elevate the overall risk of premature mortality [5].

The significance of the association between diabetes and cardiovascular pathology is undeniable and can be illustrated by the results of renowned studies, such as the Framingham Study and the Multiple Risk Factor Intervention Trial (MRFIT). Lethal outcomes in patients with diabetes are more frequently attributed to macrovascular complications rather than microvascular ones, suggesting that factors aimed at minimizing the macrovascular complications of diabetes play a decisive role in disease prognosis [6].

Given the data from a large cohort of studies assessing cardiovascular risk, contemporary endocrinology acknowledges that high and very high cardiovascular event risk is characteristic of all patients with diabetes [7]. To mitigate this risk, reducing hyperglycemia and achieving the target level of glycated hemoglobin is recommended.

Nevertheless, a subgroup of patients with satisfactory glycemic control, where the frequency of negative cardiovascular events remains elevated, has not received adequate attention. Several groups of glycemic regulation biomarkers, including insulin resistance, incretins, and indicators of glycemic variability, are considered as potential markers for assessing cardiovascular risk in these patients.

The primary pathogenetic factor in diabetes is considered to be an elevation in insulin concentration. Excessive insulin production occurs significantly earlier than hyperglycemia; however, in clinical practice, diabetes diagnosis relies solely on glucose levels and not insulin levels [8]. Researchers evaluate hyperinsulinemia and the subsequent development of insulin resistance as independent risk factors for vascular endothelial damage [9]. In turn, endothelial dysfunction may lead to cardiovascular complications through the phenomenon of pathological glycemic variability.

Moderate fluctuations in glycemia are a physiological phenomenon. For healthy individuals, their range is defined within 3.3–7.8 mmol/L. Glycemic values outside this physiological range are rarely encountered. However, in patients with diabetes, the amplitude of glycemic values significantly increases [10]. Glycemic variability (GV) is typically measured through continuous glucose monitoring; however, it can also be assessed through the concentration of carbohydrate metabolism markers, with one of the most promising being 1,5-anhydro-D-sorbitol (1,5-AG) [11].

Research has shown that 1,5-anhydro-D-sorbitol (1,5-AG) primarily enters the body through dietary sources, and its chemical structure ensures metabolic stability, with its consumption rate being equivalent to daily excretion. Renal reabsorption of 1,5-AG is 99.9%; however, there is a process of competitive inhibition of 1,5-AG reabsorption in the presence of glucosuria. Based on these findings, Japanese research groups demonstrated a reduction in the serum concentration of 1,5-AG in patients with hyperglycemia compared to those with normal carbohydrate metabolism.

Thus, 1,5-AG may serve as a valuable marker for assessing short-term glycemic fluctuations, as it has the ability to reflect not only glycemic control but also its excursion. It may hold clinical significance in evaluating treatment effectiveness by reflecting postprandial glycemia in diabetes [12-13].

Some researchers link the pathogenetic mechanisms of glycemic variability to fluctuations in insulin levels in individuals with diabetes. However, the mechanism of glycemic variability appears to be a more intricate process than the sole influence of hyperinsulinemia [14].

Glycemic fluctuations are influenced not only by insulin but also by incretin hormones, which demonstrate potential cardioprotective effects in patients with type 2 diabetes (T2D). Studies indicate a positive impact of glucagon-like peptide-1 (GLP-1) receptor agonists on cardiovascular mortality and renal outcomes in patients with T2D [15].

Considering these findings, the investigation of glycemic variability, insulin resistance, and incretin response in individuals with impaired carbohydrate metabolism and negative cardiovascular events can be deemed a promising area of research [16].

**Research aim:** To investigate markers reflecting glycemic variability, insulin resistance, and counterregulatory response in the risk assessment of cardiovascular events among patients with type 2 diabetes and prediabetes.

**Research оbjectives:**

**Research objective 1:** To evaluate the concentrations of glucose and glycated hemoglobin and assess the association of these parameters with the probability of cardiovascular events in patients with type 2 diabetes who have achieved target glycemic levels.

**Research objective 2:** To assess the level of glycemic variability reflected by 1,5-AG and the probability of developing cardiovascular events in patients with type 2 diabetes.

**Research objective 3:** To investigate indicators of insulin resistance and counterregulatory response and their relationship with 1,5-AG in patients with type 2 diabetes with and without cardiovascular events.

**Research objective 4:** To assess the level of glycemic variability reflected by 1,5-AG and the probability of developing cardiovascular events in patients with prediabetes.

**Research objective 5:** To investigate indicators of insulin resistance and counterregulatory response and their relationship with 1,5-AG in patients with prediabetes with and without cardiovascular events.

**Scientific originality**

1. For the first time, a comprehensive assessment of carbohydrate metabolism regulatory biomarkers, including indicators of insulin resistance, counterregulatory response, and 1,5-anhydro-D-sorbitol (1,5-AG) concentration, was provided in patients with type 2 diabetes with non-fatal cardiovascular events and without.
2. The concentration of 1,5-AG was investigated for the first time, establishing a connection between 1,5-AG and the development of cardiovascular events in patients with prediabetes.
3. A logistic regression model was calculated for the first time to assess the probability of cardiovascular events in patients with type 2 diabetes, considering the concentration of 1,5-AG and the homeostatic model assessment of insulin resistance (HOMA-IR) index.
4. A logistic regression model was calculated for the first time to assess the probability of cardiovascular events in patients with prediabetes, considering the concentration of 1,5-AG and glucagon-like peptide-1.
5. Threshold values for the concentration of 1,5-AG associated with the development of cardiovascular events in patients with type 2 diabetes and prediabetes were determined for the first time.

**Main provisions submitted for defense**

1. A reduction in the level of 1,5-anhydro-D-sorbitol (1,5-AG) increases the likelihood of developing cardiovascular events in patients with type 2 diabetes who have achieved the target level of glycated hemoglobin. The risk factor for non-fatal cardiovascular events in patients with type 2 diabetes is a 1,5-AG level below 353.11 μmol/L.
2. In patients with type 2 diabetes, an increase in the likelihood of cardiovascular events, along with a decrease in the concentration of 1,5-AG, is associated with an elevation in the homeostatic model assessment of insulin resistance (HOMA-IR) index.
3. A decrease in the level of 1,5-AG in patients with prediabetes increases the chances of developing cardiovascular events. The risk factor for non-fatal cardiovascular events in patients with prediabetes is a 1,5-AG level below 413.03 μmol/L.
4. In patients with prediabetes, an increase in the likelihood of cardiovascular events, along with a decrease in the concentration of 1,5-AG, is associated with an elevation in the concentration of glucagon-like peptide-1.

**Practical relevance**

1. The determination of 1,5-anhydro-D-sorbitol (1,5-AG) concentration and homeostatic model assessment of insulin resistance (HOMA-IR) index in patients with type 2 diabetes, and 1,5-AG and glucagon-like peptide in patients with prediabetes, should be considered as promising predictors for the development of non-fatal cardiovascular events.
2. The developed model for calculating the probability of cardiovascular events, considering glycemic variability control using 1,5-AG, HOMA-IR index, glucagon-like peptide-1 (GLP-1), will enhance therapeutic interventions and prevent the development of cardiovascular events in patients with carbohydrate metabolism disorders.

 **Implementation into practice**The data obtained from conducted clinical studies have been implemented and applied in the work of the prevention departments of three outpatient clinics in Karaganda: JYSAN MED LLP, Hippocrates, and the city emergency medical service center of Karaganda.

 **Author's Personal Contribution**

The dissertation candidate conducted the selection and clinical examination of patients, determined glucose and glycated hemoglobin levels using portable devices, and collected and transported biological material to the laboratory. The doctoral candidate analyzed the concentration of 1,5-anhydro-D-sorbitol (1,5-AG) using HPLC under the guidance of a research fellow from the Institute of Life Sciences at the Medical University of Karaganda Marchenko A.B. The candidate formed and populated the database of the study subjects, performed statistical data processing, and analyzed and interpreted the obtained results.

**Connection of the dissertation with other research works**

The dissertation was conducted under the auspices of the Department of Internal Medicine, Institute of Life Sciences, at the National Medical University of Karaganda within the framework of the scientific and technical program O.0769 "Development of Scientific Foundations for Creating a Preventive Environment for Public Health Preservation" of the Ministry of Healthcare of the Republic of Kazakhstan (program-targeted financing) implemented in 2017–2019 (Registration number: 0117RK00018).

**Approbation of results**

The main provisions and results of the research were presented at the International Conference "Advanced Technology and Treatment of Diabetes" (2018, Austria, Vienna); the International Scientific and Practical Conference "International Scientific Conference - 2019. Health. Science. Technology" (2019, Karaganda); the International Congress "World Congress Insulin Resistance Diabetes & Cardiovascular Disease" (2020, Los Angeles, California, USA); and at an extended meeting of the Department of Internal Medicine at the National Medical University of Karaganda.

**Publications on the topic of the dissertation work**

n the topic of the dissertation, 10 printed works have been published: among them, 2 articles in international scientific publications indexed in the Scopus database (journal "Open Access Macedonian Journal of Medical Sciences"); 3 articles in scientific publications published in journals recommended by the Committee for Control of Social and Natural Risks (journal "Astana medical journal" and "Journal of Clinical Medicine of Kazakhstan"); 4 abstracts in the proceedings of international conferences, and 1 abstract in the proceedings of a republican conference.

**Scope and structure of the dissertation**

The dissertation research is presented in 94 pages and consists of the following sections: introduction, literature review, 5 chapters describing the results of the original research, conclusions, and findings. The bibliography includes 155 literary sources. The dissertation includes 12 figures, 14 tables, and is supplemented with 2 appendices.

**Materials and research methods**

The patient recruitment was conducted from June to November 2018 at Outpatient Clinic No. 1 in Karaganda. The research protocol was approved by the Bioethics Committee of the National Medical University "Karaganda Medical University" No. 62 dated June 18, 2018. An observational cross-sectional study of a case-control type was carried out. The total number of participants was 301 individuals.

All participants were categorized into four groups for subsequent pairwise comparisons, as there were different working hypotheses for patients with diabetes and prediabetes. Patients in the first group (type 2 diabetes and cardiovascular events) were compared with patients in the second group (type 2 diabetes without cardiovascular events). Patients in the third group (prediabetes with cardiovascular events) were compared with patients in the fourth group (prediabetes without cardiovascular events).

**Study design**

**Research Methods:**

Questionnaire

Clinical examination, blood sampling with subsequent determination of glycemic regulation biomarkers

**Inclusion criteria**

Verified diagnosis of type 2 diabetes/pre-diabetes in combination with negative cardiovascular events, including non-fatal myocardial infarction and non-fatal stroke.

Patients with type 2 diabetes/pre-diabetes and risk factors for cardiovascular diseases, including arterial hypertension, abdominal obesity, and dyslipidemia, without a history of cardiovascular events.

**Exclusion criteria**

Pregnant women, individuals with severe mental and oncological disorders, and patients with type 1 diabetes are excluded from the study.

**Ethical approval**

The research protocol was developed in accordance with the ethical principles of scientific research outlined in the Helsinki Declaration by the World Medical Association. Research Protocol No. 62 was approved on June 18, 2018, by the Ethics Committee of the Karaganda State Medical University, Karaganda, Kazakhstan.

The survey was conducted based on a developed questionnaire consisting of the following sections: general and demographic information, behavioral risk factors (sufficient consumption of vegetables and fruits, physical activity), and a history of diabetes and arterial hypertension. Clinical examination included patient examination, measurement of blood pressure (BP), body mass index (BMI), and waist circumference. The study of carbohydrate metabolism regulation parameters involved determining fasting blood glucose levels, HbA1C, 1.5-AG, insulin, and the homeostatic model assessment of insulin resistance (HOMA-IR).

The examination of lipid metabolism parameters included determining total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and HbA1C. Insulin resistance indices and the incretin part of the counterregulatory response were determined using multiplex immunoassay analysis with XMap technology on Bioplex 3D. The concentration of 1.5-AG in blood plasma was determined using high-performance liquid chromatography with mass spectrometry.

All values were presented either as the median with interquartile range or as the mean ± standard deviation, depending on the normal distribution verification using the Kolmogorov-Smirnov test.

To assess correlations, the Pearson correlation coefficient was used for normally distributed data, and the Spearman rank correlation coefficient was used for non-normally distributed data. Cluster analysis was performed using the K-means clustering method. Considering the linkage coefficient in the hierarchical cluster analysis protocol, the optimal number of clusters was determined as 2. All variables included in the analysis were quantitative. Binary logistic regression was conducted depending on the data type and research questions posed. The significance level was set at p < 0.05. Sensitivity and specificity were calculated, and the quality of the model was judged by the area under the ROC curve. Statistical analyses were performed using SPSS Statistical Software 21.0 (SPSS Inc., Chicago, Illinois).

**Conclusions:**

**Conclusion 1**

The blood glucose concentration is significantly higher in patients with type 2 diabetes and cardiovascular events who have achieved the target level of glycated hemoglobin (Me1=9.81, Q25;Q75 7.7-14.8; Me2=8.55, Q25;Q75 6.72-10.07, p=0.012). However, it is not a significant factor influencing the probability of these events (OR 0.971, 95% CI 0.847-1.113, p=0.448). The concentration of glycated hemoglobin does not show significant differences between patients with type 2 diabetes with and without cardiovascular events (Me1=8.2, Q25;Q75 7.8-9.05; Me2=7.9, Q25;Q75 6.9-9.8, p=0.237) and is also not a significant factor in the probability of these events (OR 1.139, 95% CI 0.578-2.242, p=0.707).

**Conclusion 2**

The concentration of 1,5-AG in patients with type 2 diabetes and cardiovascular events is lower than in patients without cardiovascular events (Me1=215.8, Q25;Q75 186.4–280.8; Me2=275.8, Q25;Q75 233.3-350.3, p<0.001). The reduction in the concentration of 1,5-AG is associated with an increased probability of developing cardiovascular events in patients with type 2 diabetes (OR=2.272, 95% CI 2.153-2.331, p<0.001).

**Conclusion 3**

In patients with type 2 diabetes and cardiovascular events, a correlation was observed between the insulin resistance index HOMA-IR and 1,5-AG (r=0.36, p<0.001), glucagon-like peptide-1 (r=0.42, p<0.001), glucose-dependent insulinotropic polypeptide (r=0.39, p<0.001), and glucagon (r=0.56, p<0.001). The model of cardiovascular event development, adjusted for confounders such as gender, age, glucose level, and glycated hemoglobin, demonstrated an increased risk of cardiovascular events with a decrease in the concentration of 1,5-AG (adjusted OR=3.217, 95% CI 2.576-4.132, p=0.023) and an increase in HOMA-IR (adjusted OR=2.284, 95% CI 1.197-2.654, p=0.043). The concentration of 1,5-AG (AUC 0.810), as well as HOMA-IR (AUC 0.616), showed high diagnostic significance in the development of cardiovascular events.

**Conclusion 4**

In patients with prediabetes and cardiovascular events, a decrease in the concentration of 1,5-AG was observed compared to patients without cardiovascular events (Median 3=220.5, Q25;Q75 141.5–378.3; Median 4=314.6, Q25;Q75 250.8–415.1, p=0.015). In patients with prediabetes, 1,5-AG had a higher concentration than in patients with type 2 diabetes. A decrease in the concentration of 1,5-AG is associated with an increased probability of cardiovascular events in patients with prediabetes (OR=1.775, 95% CI 1.460-1.990, p=0.042).

**Conclusion 5**

In patients with prediabetes and cardiovascular events, a correlation was observed between HOMA-IR and 1,5-AG (r=0.287, p=0.034). The model of cardiovascular event development, adjusted for confounders such as gender, age, glucose level, and glycated hemoglobin, demonstrated an increased risk of cardiovascular events with a decrease in the concentration of 1,5-AG (adjusted OR=2.304, 95% CI 1.980-2.973, p=0.008) and an increase in the concentration of glucagon-like peptide-1 (adjusted OR=1.775, 95% CI 1.460-1.990, p=0.002). The diagnostic significance of 1,5-AG was AUC=0.521, and for glucagon-like peptide-1AUC was 0.714.

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