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"KARAGANDA MEDICAL UNIVERSITY"

**ANNOTATION**

dissertation work for the degree of Doctor of Philosophy (PhD)  
on the topic: Investigation of the probability of reducing the number of  
cardiovascular events in patients after acute myocardial infarction with dietary  
remodeling of the intestinal microbiota

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### **The relevance of research.**

Cardiovascular diseases (CVDs) firmly hold leading positions in the structure of global mortality, causing about 17.9 million deaths annually, according to the World Health Organization (WHO). This problem is of particular importance for low- and middle-income countries, where CVDs are the most common cause of both morbidity and premature mortality. Often, the first clinical manifestation of CVD is acute coronary syndrome (ACS). The epidemiological situation in the European region also causes serious concern: in 2019, 5.8 million new cases of ischemic heart disease (IHD) were registered in 57 member countries of the European Society of Cardiology (ESC), with CVDs accounting for about 2.2 million deaths among women and 1.9 million among men annually in these countries.

In the Republic of Kazakhstan, the problem of CVD is particularly acute and is characterized by negative dynamics. Over the ten-year period from 2008 to 2018, the prevalence of diseases of the circulatory system in the country increased by almost 2.5 times, reaching 2755.4 cases per 100,000 population. In 2021, the national average mortality rate from diseases of the circulatory system was 163.14 per 100,000 people; however, in some regions, such as the Karaganda region, it reached a critical level of 325.47 – the highest in the republic. Data for 2023 confirm the ongoing tension: the overall incidence of CVD was 2781.3 cases per 100,000 people, of which 122.1 cases were acute myocardial infarction (AMI). In the Karaganda region, the incidence of AMI was even higher – 145.3 cases per 100,000 population. Complications of CVD, based on atherosclerotic processes and arterial thromboses, continue to show an upward trend.

Diet plays a fundamental role in regulating both the composition of gut microbiota and the levels of trimethylamine-N-oxide (TMAO). TMAO, a gut microbiota metabolite, is associated with an increased risk of major adverse cardiovascular events (MACE) in patients after ST-segment elevation myocardial infarction (STEMI). TMAO is formed from dietary precursors (choline, L-carnitine, phosphatidylcholine) that are metabolized by gut bacteria such as *Clostridium* and *Proteobacteria* into TMA, which is then oxidized in the liver by the enzyme flavin-containing monooxygenase 3 (FMO3). The link between TMAO and cardiovascular disease (CVD) was first established by Hazen et al. in 2011, when elevated TMAO levels were observed in patients with atherosclerosis. Large cohort studies have confirmed that TMAO predicts the risk of myocardial infarction, stroke, and major adverse cardiovascular events (MACE), with hazard ratios (HRs) ranging from 1.1 to 1.5. TMAO activates platelets, increasing their adhesion by 30%, and enhances endothelial inflammation through the activation of the NLRP3 inflammasome.

Mouse experiments have shown that a diet rich in L-carnitine raises TMAO levels and increases atherosclerotic plaque size by 40%. The mechanisms of TMAO action are diverse. Firstly, it promotes cholesterol accumulation in macrophages, transforming them into foam cells—a key step in atherogenesis. Secondly, TMAO inhibits reverse cholesterol transport by reducing hepatic ABC transporter expression by 25%. Thirdly, it activates ROS-dependent signaling pathways, thereby increasing oxidative stress and fibrosis. In patients with coronary artery disease (CAD), TMAO levels correlate with the severity of atherosclerosis ( $r = 0.4$ –

0.6) and the risk of MACE. After STEMI, a TMAO level above 6  $\mu\text{mol/L}$  doubles the risk of death within 5 years. In this dissertation, Kaplan–Meier curves demonstrate the association between TMAO and MACE incidence, underscoring its prognostic value.

The role of TMAO is not unequivocal. Schiattarella et al. noted that its levels depend on diet: vegetarians have TMAO levels 50% lower due to reduced L-carnitine intake. Genetic polymorphisms in FMO3 can increase TMAO levels by 20–30%, contributing to interindividual variability. Environmental factors such as air pollution (PM<sub>2.5</sub>) can increase TMAO by 10% by altering the gut microbiota. Experiments with TMA-lyase inhibitors (e.g., 3,3-dimethyl-1-butanol) have shown a reduction in TMAO levels and improved cardiac function post-infarction in mice. Historically, TMAO was studied as a metabolite responsible for fishy odor (1960s), but its role in CVD has only recently become evident. Metabolomic studies have revealed a correlation between TMAO and lipid markers ( $r = 0.3$ ), highlighting its systemic impact. These findings support the potential of TMAO as both a biomarker and a therapeutic target. Experimental and clinical data have accumulated confirming that a targeted change in diet can modulate the microbiota and reduce plasma TMAO concentration. In this context, plant polyphenols such as resveratrol, curcumin, and grape polyphenols are of particular interest, being considered as promising agents for correcting dysbiosis and reducing TMAO levels. An original grape polyphenol concentrate "Qaynar" was developed in the Republic of Kazakhstan; however, its specific effect on TMAO levels, NET activity, oxidative stress status, and, consequently, on the risk of atherothrombosis and cardiovascular events in patients after AMI has not yet been studied. This determines the high relevance of the present study, aimed at investigating the potential of dietary remodeling of the intestinal microbiota using this concentrate to reduce the risk of cardiovascular complications in patients who have suffered an acute myocardial infarction.

### **Scientific Hypotheses:**

Dietary remodeling of the intestinal microbiota using the "Qaynar" grape polyphenol concentrate in patients after acute myocardial infarction (AMI) and percutaneous coronary intervention (PCI) leads to a decrease in plasma levels of trimethylamine N-oxide (TMAO), neutrophil extracellular traps (NETs), and markers of oxidative stress, which collectively contributes to reducing the risk of developing major adverse cardiovascular events (MACE).

**Aim of the Research:** To determine the potential for reducing the risk of adverse cardiovascular events in patients who have undergone acute myocardial infarction and percutaneous coronary intervention through dietary influence on the intestinal microbiota using a grape polyphenol concentrate.

### **Research Objectives:**

1. To quantitatively assess the level of free radicals and components of the antioxidant system in the blood plasma of patients with STEMI after percutaneous coronary intervention (PCI) under clinical trial conditions while taking a grape polyphenol concentrate.
2. To study the composition of the intestinal microbiome (relative abundance and alpha-diversity) and the dynamics of TMAO levels in patients with STEMI after PCI during dietary intervention using a grape polyphenol concentrate.
3. To establish the relationship between the dynamics of TMAO levels, parameters of oxidative and antioxidant status of blood plasma, and the degree of atherosclerotic lesions of coronary arteries in patients with STEMI after PCI.
4. To determine the probability of reducing the risk of major adverse cardiovascular events (MACE: death, stroke, recurrent MI, CABG, CHF) in patients with STEMI after PCI when applying dietary intervention with a grape polyphenol concentrate.
5. To assess the level of neutrophil extracellular traps (NETs) in the blood plasma of patients with STEMI after percutaneous coronary intervention during dietary intervention with a grape polyphenol concentrate and to establish their relationship with TMAO levels, oxidative stress markers, and the risk of major adverse cardiovascular events (MACE).

### **Theoretical and Practical Significance of the Dissertation:**

1. The role of trimethylamine N-oxide (TMAO) and intestinal microbiota in the development of recurrent cardiovascular events after ST-segment elevation myocardial infarction (STEMI) has been established, which complements the theory of atherothrombosis. A methodology for assessing MACE risk based on TMAO levels has been developed and implemented in clinical practice.
2. It has been proven that TMAO correlates with oxidative stress ( $r=0.454$ ,  $p<0.0049$ ) and atherosclerosis ( $r=0.569$ ,  $p<0.01$ ), deepening the understanding of vascular damage. This allowed for the justification of reducing MACE risk ( $OR=0.524$ ,  $p<0.05$ ) by correcting these factors.
3. The influence of grape polyphenols on reducing the Firmicutes/Bacteroidetes ratio ( $p<0.021$ ) and TMAO (by 25%,  $p<0.01$ ) has been theoretically confirmed. This has been practically implemented through the use of the "Qaynar" concentrate as a supplement to STEMI therapy.
4. The results expand knowledge about the "gut-heart" axis and have been implemented in the cardiology department of the "Regional Medical Center" KGP in Karaganda, improving the long-term prognosis of patients after STEMI and percutaneous coronary intervention.
5. The research creates a basis for further developments in the field of diet therapy for CVD, and its materials are integrated into the educational programs of the "Karaganda Medical University" NAO, contributing to specialist training.

### **Scientific Novelty of the Research Results:**

1. For the first time, a reduction in MACE risk through dietary intervention has been proven: a clinical study on patients after STEMI and PCI demonstrated that the use of the "Qaynar" grape polyphenol concentrate reduces the probability of major adverse cardiovascular events (OR=0.524,  $p<0.05$ ) by remodeling the intestinal microbiota and reducing TMAO levels.
2. A new quantitative link between TMAO and atherosclerosis has been established: a significant correlation was found between plasma TMAO levels and the degree of atherosclerotic lesions of coronary arteries according to the SYNTAX Score ( $r=0.569$ ,  $p<0.01$ ), which clarifies the role of TMAO as a risk biomarker in patients after STEMI.
3. An original approach to microbiota correction has been developed: it was shown for the first time that grape polyphenols alter the composition of the intestinal microbiota, reducing the Firmicutes/Bacteroidetes ratio ( $p<0.021$ ) and TMAO production (by 25%,  $p<0.01$ ), which represents a new mechanism for dietary prevention of CVD (Certificate of State Registration of Rights to an Object of Copyright No. 55196 dated February 26, 2025).
4. The dynamics of oxidative stress under the influence of polyphenols have been identified: it was established that a decrease in TMAO levels is accompanied by a reduction in oxidative stress markers (MDA,  $r=0.454$ ,  $p<0.0049$ ; HIF1 $\alpha$ ,  $r=0.321$ ,  $p<0.031$ ) and an increase in antioxidant potential (QT,  $p<0.05$ ) in patients after STEMI, which has not been previously described in this context.
5. A methodology for predicting MACE risk based on TMAO dynamics has been developed and registered, first applied in clinical practice for risk stratification in patients with STEMI (Certificate of State Registration of Rights to an Object of Copyright No. 57415 dated April 28, 2025).

### **Main Provisions for Defense:**

1. Dietary intervention using the 'Qaynar' grape polyphenol concentrate in patients with STEMI after PCI reduces the level of free radicals (MDA by 18.2%,  $p=0.003$ ) and increases antioxidant potential (QT by 25.6%,  $p<0.005$ ), which was demonstrated for the first time in Kazakhstan.
2. Dietary intervention using the 'Qaynar' grape polyphenol concentrate in patients with STEMI after PCI leads to remodeling of the intestinal microbiota (reduction of Firmicutes/Bacteroidetes ratio by 25.3%,  $p=0.002$ ; increase in alpha-diversity by 15.2%,  $p=0.017$ ) and a decrease in TMAO levels by 28.6% ( $p<0.001$ ), which was demonstrated for the first time in Kazakhstan.
3. TMAO levels in patients with STEMI after PCI are significantly associated with the severity of atherosclerosis (SYNTAX Score I,  $r=0.351$ ,  $p=0.002$ ) and oxidative status (MDA,  $r=0.392$ ,  $p=0.008$ ; QT,  $r=-0.315$ ,  $p=0.017$ ), and its reduction under the influence of 'Qaynar' contributes to the regression of atherosclerotic lesions ( $\beta=-0.321$ ,  $p=0.012$ ).
4. The intake of 'Qaynar' grape polyphenol concentrate by patients with STEMI after PCI reduces the risk of MACE by 45% (HR=0.55,  $p=0.043$ ) and the frequency

of MACE to 15.6% versus 26.0% in the control group ( $p=0.042$ ), which is associated with a decrease in TMAO ( $OR=0.42$  at  $\leq 4.5 \mu\text{mol/L}$ ,  $p=0.030$ ).

5. Dietary intervention with 'Qaynar' in patients with STEMI after PCI reduces NET levels (by 2.3%,  $p=0.899$ ), which correlate with TMAO ( $r=0.412$ ,  $p=0.005$ ) and MACE risk ( $r=0.298$ ,  $p=0.047$ ), thereby reducing cardiovascular risk ( $OR=0.524$ ,  $p=0.014$ ).

### **Approbation of the Work**

The research topic was approved by the Bioethics Committee of the "Karaganda Medical University" NAO (Protocol No. 6 dated February 7, 2022,) (Appendix A), the Local Bioethics Commission of the "Karaganda Medical University" NAO (Protocol No. 2 dated September 20, 2022, assigned number No. 2), and by decision dated September 24, 2020, No. 05-2020, of the Local Ethics Commission of NLA Nazarbayev University (Appendix B).

The results of the conducted research were reported at scientific and practical conferences:

- 15th Congress of the European Association for Clinical Pharmacology and Therapeutics (EACPT), Athens, Greece 2022: Dietary remodeling of intestinal microbiota to reduce the number of cardiovascular events in patients after acute myocardial infarction;
- II International Forum of Genomic and Biomedical Technologies "From Birth to Active Longevity," Surgut, 2022;
- VII International Scientific and Practical Forum "Shypazhai-2023," Borovoe;
- X Republican Scientific and Practical Conference "Multifaceted Cardiology. Successes and Unresolved Issues." Astana 2023

### **Information on Publications**

- 6 scientific works have been published on the topic of the dissertation, including:
  - 3 in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan;
  - 2 in journals indexed in the Scopus database;
  - 1 abstracts published in the materials of international conferences;
  - 2 certificates of entry of information into the state register of rights to objects protected by copyright (Appendix G).

### **Research Materials and Methods**

The study was conducted as a prospective clinical trial at the cardiology department of Karaganda's "Multidisciplinary Hospital No. 2" from 2021 to 2023. The object of the study was the "Qaynar" biologically active food supplement – a concentrate of dark grape varieties polyphenols of Kazakh selection (Cabernet Sauvignon, Saperavi) with a polyphenol content of at least 20 mg/ml. The study

design was approved by the Bioethics Committee of the "Karaganda Medical University" NAO (Protocol No. 6 dated 07.02.2022, et al.) and the Local Ethics Commission of NLA Nazarbayev University (decision dated 24.09.2020, No. 05-2020), and was conducted in accordance with the principles of the Declaration of Helsinki.

The object of research was the "Qaynar" biologically active food supplement (certificate of state registration of products KZ 16.01.98.003.R.001043.11.21 dated November 2, 2021) – a concentrate of polyphenols from dark grape varieties of Kazakh selection with a polyphenol content of at least 20 mg/ml.

The subject of research was the dynamics of the composition and metabolic activity of the intestinal microbiota, plasma levels of trimethylamine N-oxide (TMAO) and neutrophil extracellular traps (NETs), indicators of oxidative stress and antioxidant status, the degree of atherosclerotic lesions of coronary arteries, as well as the frequency and predictors of major adverse cardiovascular events (MACE) in patients with ST-segment elevation myocardial infarction (STEMI) after percutaneous coronary intervention (PCI) during dietary intervention with the "Qaynar" grape polyphenol concentrate.

The study included 95 patients aged 30 to 75 years with STEMI who had undergone successful PCI. Patients were randomized by the double-envelope method into two groups: the main group (n=45) received the "Qaynar" grape polyphenol concentrate at 15 ml twice a day 30 minutes before meals for 3 months in addition to standard therapy, while the control group (n=50) received standard therapy and placebo. Patient selection was carried out according to strict inclusion criteria (STEMI after stenting, age 30-75 years, ability to comply with the protocol) and exclusion criteria (cardiogenic shock, ineffective stenting, chronic/acute intestinal diseases, grape allergy, oncological diseases, etc.). All participants signed informed consent.

Comprehensive examination of patients was carried out at three time points: baseline (at admission), after 3 months (at the end of the dietary intervention course), and after 12 months (endpoint for assessing the frequency of MACE). The following parameters were assessed:

- Clinical and demographic data and risk factors.
- Intestinal microbiota composition: Stool samples were analyzed by sequencing the V3–V4 region of the 16S rRNA gene on the Illumina MiSeq platform; bioinformatics data processing was performed using QIIME 2 to assess taxonomic composition (relative abundance, Firmicutes/Bacteroidetes ratio) and alpha-diversity (Shannon, Simpson indices).
- Trimethylamine N-oxide (TMAO) level: Determined in blood plasma by liquid chromatography with tandem mass spectrometry (LC-MS/MS, Agilent 6470).
- Markers of oxidative stress and antioxidant status: Levels of malondialdehyde (MDA), hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), methylglyoxal (MG), advanced oxidation protein products (AOPP), acid-soluble fractions (ASF), extracellular RNA and DNA were measured in blood plasma and/or erythrocytes. Antioxidant status (fast-acting (Q1), slow-acting (Q2) antioxidants, and total charge (QT)) was assessed on the e-BQC device.

- Neutrophil extracellular traps (NETs) level: Determined in blood plasma by enzyme-linked immunosorbent assay (ELISA) for quantitative determination of myeloperoxidase (MPO) and DNA complexes.

Degree of atherosclerotic lesions of coronary arteries: Assessed angiographically using the SYNTAX Score I scale. Major adverse cardiovascular events (MACE): included all-cause mortality, non-fatal recurrent myocardial infarction, acute cerebrovascular accident (stroke), need for coronary artery bypass grafting (CABG) or repeat PCI, development or worsening of chronic heart failure (CHF); recorded during 12 months of follow-up.

Statistical data processing was performed using SPSS Statistics (version 26.0) and R (version 4.3.1) software. Methods of descriptive statistics, normality testing (Kolmogorov-Smirnov, Shapiro-Wilk tests) were applied. Student's t-test or Mann-Whitney U-test for quantitative data, and chi-square ( $\chi^2$ ) test or Fisher's exact test for qualitative data were used for group comparisons. Dynamics of indicators were assessed using paired t-test or Wilcoxon test. Relationships between variables were analyzed using Spearman or Pearson correlation coefficients. Kaplan-Meier survival analysis with a log-rank test was used to assess MACE risk. The prognostic value of biomarkers (TMAO, NETs) was assessed using ROC analysis with calculation of the area under the curve (AUC) and determination of optimal cut-off points by Youden's index. Multiple linear and logistic regression were used to identify independent predictors and assess odds ratios (OR). The level of statistical significance was set at  $p < 0.05$ .

### **Conclusions:**

1. Patients with a history of ST-segment elevation acute myocardial infarction who used the "Qaynar" grape polyphenol concentrate showed a significant decrease in oxidative stress marker levels after three months: malondialdehyde (MDA) levels decreased by 18.2% ( $p = 0.003$ ), hypoxia-inducible factor (HIF) by 14.5% ( $p = 0.012$ ), methylglyoxal (MG) by 11.3%, while there was a trend towards a decrease in neutrophil extracellular trap (NET) levels by 2.3%. The level of statistical significance was  $p = 0.899$ . Antioxidant activity significantly increased as a result of the study: Q1 by 22.4%, Q2 by 19.8%, QT by 25.6% (significance level  $p < 0.005$ ).
2. Dietary intervention with the "Qaynar" concentrate led to changes in the intestinal microbiota composition: the Firmicutes/Bacteroidetes ratio decreased by 25.3% ( $p = 0.002$ ), and trimethylamine N-oxide (TMAO) levels decreased by 28.6% ( $p < 0.001$ ). No statistically significant changes in microbiota were observed in the control group; however, TMAO levels increased by 13.8% ( $p = 0.014$ ).
3. A significant positive correlation was found between TMAO levels (correlation coefficient  $r = 0.351$ ;  $p = 0.002$ ), NETs (correlation coefficient  $r = 0.335$ ;  $p = 0.025$ ), and MDA (correlation coefficient  $r = 0.392$ ;  $p = 0.008$ ) with the severity of atherosclerotic lesions of coronary arteries according to the SYNTAX Score I scale. An inverse relationship was also found between the antioxidant potential QT and the degree of atherosclerosis (correlation coefficient  $r = -0.315$ ;  $p = 0.017$ ).

Multiple regression analysis confirms that a decrease in TMAO, NETs, and MDA levels is associated with a reduction in atherosclerotic burden ( $R^2 = 0.48$ ).

4. Dietary intervention reduced the incidence of MACE in the "Qaynar" group to 15.6% (7 cases) versus 26.0% (13 cases) in the control group ( $p=0.042$ ). The probability of MACE absence after one year was higher in the "Qaynar" group — 84.4% versus 74.0% ( $p=0.042$ ). TMAO and NETs were predictors of risk: in the "Qaynar" group, their decrease (TMAO  $\leq 4.5$   $\mu\text{mol/L}$ , NETs  $\leq 1.5$  OD) reduced MACE risk by 58% (OR=0.42,  $p=0.030$ ) and 47.6% (OR=0.524,  $p=0.014$ ), respectively, while in the control group, their increase (TMAO  $\geq 6.0$   $\mu\text{mol/L}$ , NETs  $\geq 1.5$  OD) increased the risk by 2.81 times (OR=2.81,  $p=0.006$ ) and 2.72 times (OR=2.72,  $p=0.008$ ), respectively.

5. NET levels in the "Qaynar" group showed a downward trend (by 2.3% after 3 months,  $p=0.899$ ), whereas in the control group, they increased by 174% ( $p=0.034$ ). NETs correlate with TMAO ( $r=0.412$ ,  $p=0.005$ ), MDA ( $r=0.387$ ,  $p=0.009$ ), and MACE ( $r=0.298$ ,  $p=0.047$ ), and their decrease reduces the risk of cardiovascular events (OR=0.524,  $p=0.014$ ). The prognostic value of NETs (AUC=0.78) makes them a useful biomarker for assessing inflammation and MACE risk.