RELATIONSHIP BETWEEN TRIMETHYLAMINE N-OXIDE AND TOTAL CARDIOVASCULAR RISK IN THE POPULATION OF CENTRAL KAZAKHSTAN

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ABSTRACT

Aim: To investigate relationship between the level of trimethylamine N-oxide (TMAO) and total cardiovascular risk (CVR) among the population of Central Kazakhstan.

Materials and methods: The cross-sectional investigation of urban and rural population of the Karaganda region was carried out. A total of 1,235 residents of the Karaganda region aged 18 to 65 were examined. In addition to calculating the total cardiovascular risk on the Systemic Coronary Risk Evalution (SCORE) scale, questionnaires, general laboratory tests, the level of TMAO was determined by the high performance liquid chromatography with mass-selective mass spectrometry (HPLC-MS/MS).

Results: There was a strong direct relationship between the level of TMAO and the total CVR in men ($r = 0.907$, $p < 0.001$), a moderate direct relationship in women ($r = 0.559$, $p < 0.001$). According to the results of the binary logistic regression, the OR for men is 4.348 (95% CI 1.684;11.227, $p < 0.005$), the OR for women is 1.624 (95% CI 1.408;1.837, < 0.001).

Conclusion: Thus, the relationship between elevated titers of TMAO and high total cardiovascular risk showed common pathogenetic mechanisms in its development and confirmed the diagnostic and prognostic significance of these indicators among the population of the Central Kazakhstan.

Keywords: TMAO, total cardiovascular risk, cardiomarkers, Central Kazakhstan.

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Introduction

Over the past 30 years, the prevention and treatment of cardiovascular diseases, shows positive results in the form of a trend towards a slowdown in the rate of increase in mortality worldwide[1-4]. This became possible due to the improvement of medical services (the introduction of state screening programs, the modernization of primary health care), the effective modification of risk factors (reduction in the percentage of smoking, low physical activity, control of hypertension, lipid spectrum), the introduction of modern clinical recommendations into practical public health (combined therapy with modern antihypertensive, cardioprotective, cholesterol-lowering drugs, antiplatelet agents). Despite this fact, this group of diseases remains the leading cause of death both in our country and worldwide[5-7]. In this regard, the issue of timely prevention and treatment remains urgent, which become more effective in the presence of diagnostic indicators and indicators of the effectiveness of the therapy.

In our work, these indicators were an estimate of 10 years of total cardiovascular risk with the help of the European SCORE model[8] and the use of a

new cardiomarker TMAO[9-11], reflecting one of the main links of the pathophysiological process of cardiovascular diseases (CVD) and which is a potentially modifiable risk factor.

Aim

To investigate relationship between the level of TMAO and total CVR among the population of Central Kazakhstan.

Materials and methods

One-stage cross-sectional research was carried out in the form of screening, among urban and rural population of Central Kazakhstan. A total of 1,235 Karaganda residents aged 18 to 65 years who gave informed consent to participate in the study were examined, including 364 men (29.48%), 871 women (70.52%). The deformation of the sample structure by sex is to a certain extent due to the prevalence of the female population compared to that of the male population, in addition, men more often refused to participate in the screening study and interrupted the questionnaire. From the initial sample, the formation of groups of "copy-pairs" was conducted, according to the signs: sex, age, level of total cardiovascular risk. The final sample size was 304 people, including 46 men (15.13%) and 258 women (84.87%). From the study excluded pregnant women, people with severe mental illness. Primarily, the following work was carried out with the population: questioning, anthropometry, measurement of blood pressure, determination of glucose and total cholesterol level, blood sampling for TMAO level study.

In the subsequent, was carried out the calculation of the total cardiovascular risk by SCORE scale. By results of calculations, all investigated conditionally are divided into group of low risk (SCORE <1%) and high risk (SCORE> 1%). Analysis of the TMAO content in the plasma of the subjects was carried out by HPLC-MS/MS. The methods of quantitative determination of the level of TMAO were reviewed[12], which resulted in the development of an improved method for the quantification of TMAO by the HPLC-MS/MS, characterized by the simplicity and speed of sample preparation and analysis, minimum costs, and high measurement accuracy.

The validation of the methodology was carried out in accordance with the requirements of the guidance documents, OECD/WHO on GLP, guidelines for the validation of the FDA's bioanalytical techniques, EMA. On this method was filed an application for obtaining a patent of the Republic of Kazakhstan for an invention, priority certificate No. 2017/0643.1 was received.

Statistical analysis

For statistical analysis we used IBM SPSS Statistics, Version 24. Data analysis was performed with the significance level $\alpha=0,05$. Check on the normal distribution of quantitative data was performed using the Kolmogorov-Smirnov test. Description of the quantitative data was carried out on the basis of median and quartiles. For qualitative data it was calculated the proportion of individuals with traits of interest and 95% confidence interval of the proportion calculated by Klopper-Pearson method. We used U criterion of Mann-Whitney to compare the independent samples. Relationship of cytokine levels and total cardiovascular risk was assessed using Pearson's correlation coefficient. For evaluation of the outcome prediction was used method of binary logistic regression.

Results

	n=304
Age, years	50 (45;53)
Men,%	15.13
Women,%	84.87
BMI, kg/m²	29.17 (25.36;33.58)
Systolic BP, mmHg	125 (110;140)
Diastolic BP, mmHg.	80 (70;90)
Total cholesterol, mmol/l	5.45 (4.32;6.18)
Fasting blood glucose, mmol/l	5.40 (5.10;5.90)
Arterial hypertension, %	48.03 (42.3-53.8)
Diabetes mellitus (type 1,2),%	6.91 (4.4-10.5)
Smoking,%	16.78 (12.9-21.6)
The median Me, Q25, Q75 95% CI are indicated in parentheses	

Table 1: Baseline participant characteristics.

Table 1 shows the basic characteristics of those surveyed in the general population. After the formation of the "copy-pairs" groups, the subjects were represented by both men and women, aged 32

to 65, the median age was 50 years. Among the examined, females predominated (84.87%). When evaluating the BMI, it is evident that the surveyed patients have an excess body weight of 29.17 kg/m2, with median BP 125/80 mm Hg, cholesterol 5.45 mmol/l. The prevalence of smoking in the general population was 16.78%, arterial hypertension 48.03%, diabetes mellitus (type 1 and type 2) 6.91%. Thus, among the modified risk factors, hypertension is the number one priority in terms of prevalence, then overweight, smoking, diabetes, hypercholesterolemia.

	SCORE, low risk (n=152)	SCORE, high risk (n=152)	p
Age, years	50(45;53)	50(45;53)	-
Men,%	7.57	7.57	-
Women,%	42.43	42.43	-
BMI, kg/m²	27.56 (24.14;31.18)	31.21 (27.93;34.77)	<0.001
Systolic BP, mmHg	120 (110;130)	140 (120;160)	<0.001
Total cholesterol, mmol/l	4.71 (3.87;5.56)	5.89 (5.22;6.43)	<0.001
Fasting blood glucose, mmol/l	5.30 (5.00;5.70)	5.60 (5.20;6.20)	≤0.05
Arterial hypertension, %	28.95 (21.89;36.85)	67.10 (59.03;74.50)	<0.001
Diabetes mellitus (type 1,2),%	0 (00)	13.82 (8.76;20.34)	<0.001
Smoking,%	13.82 (8.76;20.34)	19.74(13.73;26.96)	<0.001
The median Me, Q25, Q75 95% CI are indicated in parentheses			

Table 2: Basic characteristics of the participants, depending on the level of total CVR.

Table 2 shows the basic characteristics of the survey sample, depending on the level of total CVR. When comparing the general clinical and laboratory parameters between the groups of high and low total cardiovascular risk in the general population, there are statistically significant differences in all items with a degree of reliability from ≤0.05 to <0.001, which confirms the contribution of each of them to the formation of the total cardiovascular risk, cardiovascular risk.

The results of the HPLC-MS / MS blood-level analysis are shown in Table 3. When considering the results, both in the general population (3.30, Q25-2.05, Q75-4.70 and 5.90 Q25-4.30 ; Q75-8.15, p <0.001), so among men (2.70, Q25-1.50, Q75-4.40 and 6.30, Q25-5.10, Q75-8.30, p < 0.001) and women (3.30, Q25-2.15, Q75-4.75 and 5.90, Q25-4.20, Q75-8.15, p <0.001), the median of TMAO titers is significantly higher in the high-grade group of the total cardiovascular risk.

	SCORE, low risk	SCORE, high risk	p
General population	(n=152)	(n=152)	
TMAO	3.30 (2.05;4.70)	5.90 (4.30;8.15)	<0.001
Men	(n=23)	(n=23)	
TMAO	2.70 (1.50;4.40)	6.30 (5.10;8.30)	<0.001
Women	(n=129)	(n=129)	
TMAO	3.30 (2.15;4.75)	5.90 (4.20;8.15)	<0.001
The median Me, Q25, Q75 are indicated in parentheses			

Table 3: Comparison of cardiomarker indicators (μmol/l), in groups with low and high total CVR.

The scattering diagrams (Figures 1-2) show the results of the evaluation of the relationship between the level of TMAO and the total CVR.
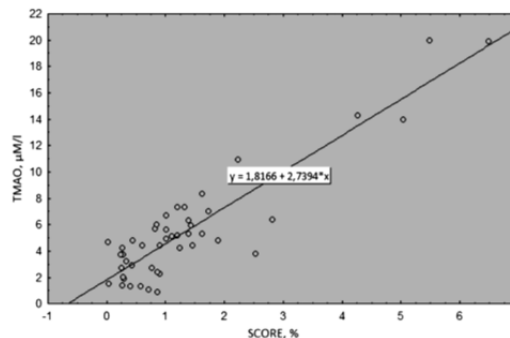
Fig. 1: Correlation of TMAO with the total CVR in the male population.
r = 0.907; p <0.001.
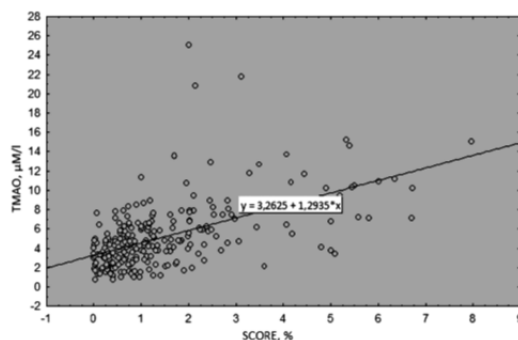
Fig. 2: Correlation of TMAO with the total CVR in the female population.
r = 0.559; p <0.001.

Based on the results of the correlation analysis, it can be seen that in both populations there are different degrees of severity, a statistically significant relationship between TMAO and total CVR, while in the male population this level is most pronounced.

To confirm this relationship, binary logistic regression analysis was additionally carried out. Based on the results of the analysis presented in Table 4, it is evident that the chances of increasing the level of TMAO in the group of high total CVR are higher than among those with normal levels of total CVR.

Total cardiovascular risk low / high			
	OR	95%CI	p
TMAO, men	4.348	1.684;11.227	< 0.005
TMAO, women	1.624	1.408;1.837	< 0.001

Table 4: Evaluation of the relationship between the levels of TMAO and total CVR in the male and female populations (the result of binary logistic regression).

Discussion

Of all the known causes of the development of most cardiovascular diseases that are amenable to therapeutic effects, atherosclerosis is one of the fundamental. Despite the use of modern lipid-lowering therapy, high residual cardiovascular risk remains[13, 14]. One of the solutions to the issue of prevention and treatment of cardiovascular diseases is the timely stratification of the risk of developing fatal cardiovascular events (CVE) among the population. This diagnostic procedure can be performed using the European Model SCORE (Systemic Coronary Risk Evaluation), which assesses the 10-year risk of developing fatal CVE associated with atherosclerosi[8].

This model was chosen by us, as it is adapted for the inhabitants of Russia and the CIS countries and is applicable to our population. Another solution for early diagnosis of CVD is the use of blood cardiomarkers that reflect most of the links in the pathophysiological process and have a diagnostic and prognostic role. For this purpose, in our study, we used a recently discovered cardiac markers trimethylamine N-oxide. TMAO is a metabolite of phosphatidylcholine and L-carnitine, which are abundant in red meat, egg yolk, milk, liver, and seafood such as mollusks, crustaceans[10, 11, 15]. This metabolite is considered as one of the markers of instability of atherosclerotic plaque leading to the development of thromboses both in the vessels of the coronary vessels and in the vessels of the brain[16, 17]. However, there is insufficient data on the relationship between the level of TMAO and the total cardiovascular risk.

On closer examination, it is evident that in the group with high total CVR the presence of all modifiable risk factors for CVD was noted: obesity, hypertension, hypercholesterolemia, smoking, diabetes mellitus. As is known, all these risk factors are associated with the acceleration of the progression of atherosclerotic vascular disease, an increase in CVD mortality[18-21]. When assessing the level of the cardiomarker, the median level of TMAO in the group with low total CVR does not exceed the reference values, while significantly higher in the group with high total CVR[9]. To assess the relationship between the level of TMAO and total CVR, a correlation analysis was performed showing a statistically significant strong direct relationship in men (r = 0.907, p <0.001) and a moderate direct relationship in women (r = 0.559; p <0.001).

Based on the results of an additional binary logistic regression analysis, the chances of increasing the level of TMAO in the group of high total cardiovascular risk are higher than among those with normal levels of total CVR, 4.34 times for men and 1.62 times for women. It is worth noting that men have a higher level of correlation and odds ratio between the level of TMAO and the total cardiovascular risk, this can be explained by the fact that men more often and more consume red meat, and also because male sex is an additional unmodifiable factor risk of CVD.

Conclusion

Thus, the relationship between elevated titers of TMAO and high total cardiovascular risk showed common pathogenetic mechanisms in its development and confirmed the diagnostic and prognostic significance of these indicators among the residents of the Central Kazakhstan.

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