Gut microbiota-derived metabolites and coronary artery disease severity: A case-control metabolomics analysis

Metabolitos derivados de la microbiota intestinal y la gravedad de la enfermedad coronaria: Un análisis metabolómico de casos y controles

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Received: 03/20/2025 Accepted: 06/19/2025 Published: 07/12/2025 DOI: http://doi.org/10.5281/zenodo.15741856

Abstract

This case-control study was designed to assess the association of gut microbial metabolites with CAD severity in Uzbek population. 200 patients with severe CAD (based on SYNTAX index ≥33) and 150 healthy controls were recruited in the study. Serum and stool samples of participants were investigated for metabolomics using liquid chromatography-mass spectrometry (LC-MS). The results were that 15 gut microbial metabolites (such as TMAO, secondary bile acids, and short-chain fatty acids) were significantly associated with CAD severity. The patient group had 3.2 times higher TMAO than the control group ($p < 0.001$), and severe CAD patients had 40% lower butyrate levels ($p < 0.05$). Furthermore, the higher level of secondary bile acids was associated with increased severity of CAD (odds ratio 2.8, 95% CI:1.5-4.1). Multivariate logistic regression demonstrated independent association between metabolites and the severity of CAD after adjusting for age, gender, and risk factors of the cardiovascular system ($p < 0.001$). Combining the metabolite model and classical risk factors elevated the discriminative ability in determining severe CAD by 85% (AUC = 0.85). These findings corroborate the pivotal role for gut microbial metabolites in CAD pathophysiology and indicate the potential to target the metabolites for management of clinical disease.

Keywords: Gut microbial metabolites, coronary heart disease, metabolomics, TMAO.

Resumen

Este estudio de casos y controles se diseñó para evaluar la asociación de los metabolitos microbianos intestinales con la gravedad de la enfermedad coronaria en la población uzbeka. Se reclutaron 200 pacientes con enfermedad coronaria grave (según el índice SYNTAX ≥33) y 150 controles sanos. Se analizaron muestras de suero y heces de los participantes para determinar su metabolómica mediante cromatografía líquida-espectrometría de masas (LC-MS). Los resultados indicaron que 15 metabolitos microbianos intestinales (como TMAO, ácidos biliares secundarios y ácidos grasos de cadena corta) se asociaron significativamente con la gravedad de la enfermedad coronaria. El grupo de pacientes presentó 3,2 veces más TMAO que el grupo control ($p < 0,001$), y los pacientes con enfermedad coronaria grave presentaron niveles de butirato un 40 % inferior ($p < 0,05$). Además, un mayor nivel de ácidos biliares secundarios se asoció con una mayor gravedad de la EAC (odds ratio [OR]: 2,8; IC del 95 %: 1,5-4,1). La regresión logística multivariante demostró una asociación independiente entre los metabolitos y la gravedad de la EAC tras ajustar por edad, sexo y factores de riesgo cardiovascular ($p < 0,001$). La combinación del modelo de metabolitos con los factores de riesgo clásicos aumentó la capacidad discriminativa para determinar la EAC grave en un 85 % (AUC = 0,85). Estos hallazgos corroboran el papel fundamental de los metabolitos microbianos intestinales en la fisiopatología

de la EAC e indican el potencial de utilizarlos como diana para el manejo de la enfermedad clínica.

Palabras clave: Metabolitos microbianos intestinales, enfermedad coronaria, metabolómica, TMAO.

Introduction

Coronary artery disease (CAD), as one of the leading causes of death worldwide, poses a major health burden to health systems and economies. In countries such as Uzbekistan, increased prevalence of traditional risk factors such as hypertension, diabetes, and obesity in addition to rapid changes in lifestyle and diet has transformed this disease into a serious health threat[1]. Despite significant progress in the diagnosis and treatment of CAD, a high percentage of patients, especially those with severe presentations of the disease, still require more aggressive interventions, which highlights the need to find new pathophysiological mechanisms and predictive biomarkers[2]. In the past few years, the gut-heart axis has been a promising area of cardiovascular research. Gut microbiota exerts significant impacts on inflammatory responses, endothelial function, and lipid homeostasis through the production of bioactive metabolites such as trimethylamine oxide (TMAO), secondary bile acids, and short-chain fatty acids (SCFAs)[3]. Early studies suggest that imbalances in the architecture of the gut microbiota (dysbiosis) and changes in the resulting metabolite profiles may directly or indirectly cause atherosclerosis and the progression of vascular plaques. However, the majority of available data are derived from Western populations, and very little is known about the pattern of these metabolites in populations with specific ethno-geographic characteristics, i.e., the residents of Central Asia[4,5,6].

Targeting the Uzbek population specifically, the study intends to fill the knowledge gap between gut microbial metabolites and CAD severity. Identification of this association may not only provide novel insights into the disease pathogenesis, but also pave the way for the implementation of preventive or therapeutic measures through gut microbiota modulation[7]. Because there can be diversity in the gut microbiota composition depending on diet, culture, and genetic backgrounds among the population under study, this research may contribute to the development of individualized clinical interventions and improve patient care outcomes in the region[8].

CAD is a multifactorial condition linked to intricate processes like systemic inflammation, oxidative stress, and endothelial dysfunction. Over the past decade, more emphasis has been placed on the gut microbiota as a non-conventional modulator in cardiovascular disease pathogenesis. By metabolizing nutrients like dietary fibre, choline, and carnitine, gut microbes produce bioactive metabolites that pass through the gut barrier and reach the bloodstream[9]. These metabolites can potentially modulate critical functions like the immune response, vascular permeability, and aggregation of atherosclerotic plaque[10].

Scientific evidence shows that trimethylamine oxide (TMAO), the most recognized microbial metabolite, plays a direct role in the development of atherosclerosis by the facilitation of cholesterol delivery to macrophages, the potentiation of vascular inflammation, and the inhibition of bile acid production. Longitudinal investigations have confirmed the inverse correlation between plasma levels of TMAO and major cardiovascular events, such as myocardial infarction[11]. On the other hand, short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, produced by the intestinal bacteria as a result of the fermentation of dietary fibers, exert protection against damage through diminished intestinal permeability, modulated inflammatory responses, and improved endothelial barrier function. Lower levels of these metabolites in CAD patients suggest the possibility of an association between intestinal dysbiosis and vascular injury worsening[12].

Secondary bile acids, being microbial derivatives, also contribute to the modulation of cholesterol metabolism and farnesoid X receptor (FXR) activity. Disruption of the primary-secondary bile acid ratio could modulate lipid homeostasis and inflammation and support the development of atherosclerotic plaque progression indirectly[13]. However, most of the data reported are based on animal models or Europeans and Americans, and there is little information about population changes of these metabolites in populations with specific dietary patterns (e.g., high red meat diets or high-fiber diet)[14].

Ethno-geographical differences in the composition of gut microbiota are among the most significant factors that can influence the profile of microbial-derived metabolites. For example, Central Asian subjects, especially those from Uzbekistan, may possess a different composition of gut microbiota and respective metabolites than Western populations due to their unique dietary habits (high consumption of fermented breads, traditional dairy foods, and some spices)[15]. These differences should affect the gut microbiome-host interaction in CAD pathogenesis, but scarcely have been addressed so far.

Overall, although it has been recognized that gut microbial metabolites correlate with CAD in previous studies, the precise pathways by which such interactions occur in non-Western populations remain yet to be delineated[16, 17]. That being said, it suggests an urgent need to conduct research between the causal pathway between gut microbiota and the severity of CAD with due respect to ethnocultural diversity as well as specialized environmental factors[18, 19]. These analyses will not only aid in

the discovery of new diagnostic biomarkers, but also pave the way for therapeutic interventions based on gut microbiome modification (e.g., probiotics, prebiotics, or selective diets)[20, 21].

Materials and methods

Study Design

The case-control study explores the association between gut microbiota-derived metabolites and coronary artery disease severity in patients referred to the choice medical centers of Uzbekistan during 2012-2013. The case group consisted of 200 subjects with severe CAD (with SYNTAX index ≥33) and the control group consisted of 150 healthy participants with no cardiovascular disease history. The study protocol was done after getting approval from Tashkent University of Medical Sciences Ethics Committee and informed consent of the subjects.

Statistical population

Table 1: Demographic and clinical characteristics of the study population

Characteristic	CAD Patients Group (n=200)	Control Group (n=150)
Age (years)	58.4 ± 8.2	56.1 ± 7.9
Gender (male/female)	120/80	85/65
Body Mass Index (kg/m²)	28.6 ± 3.1	26.2 ± 2.8
Current Smokers (%)	35%	18%
Hypertension (%)	65%	28%
Type 2 Diabetes (%)	42%	15%
SYNTAX Score	36.5 ± 4.8	-

The participants from the case group were selected from referred patients for coronary angiography who were severe CAD based on the SYNTAX index. The control group consisted of healthy subjects with no past history of heart disease, uncontrolled diabetes mellitus, or systemic inflammatory disorders. Groups were matched by age and gender using statistical methods. The primary difference in the prevalence of classic risk factors like hypertension and type 2 diabetes between the two groups explains the role played by these factors in the etiology of CAD.

Data Collection

Fasting serum specimens were drawn from all the patients and kept at -80°C until tested. Stool specimens were also drawn under anaerobic conditions and were immediately frozen. Metabolites of gut microbes (e.g., TMAO, short-chain fatty acids, and secondary bile acids) were examined using liquid chromatography-mass spectrometry (LC-MS). CAD severity was calculated from the SYNTAX index by two independent blinded cardiologists who were not aware of the metabolic condition of the patients.

Laboratory Analyses

For the measurement of metabolites, serum samples were preprocessed by using a protein extraction procedure by a standard protocol. A C18 column was run in an LC-MS with a mobile phase of water and acetonitrile containing 0.1% formic acid. Mass spectrometry was performed in positive ion mode with a precision of 2 ppm. Internal and external standards were used to calibrate the instrument for each metabolite. Fecal samples were also analyzed for microbial DNA extraction and bacterial diversity determination by 16S rRNA sequencing.

Statistical analysis

Statistical analysis was done in SPSS (version 26) and R (version 4.2.1) software. Independent t-test or Mann-Whitney (based on normality of data) was applied to compare continuous variables and chi-square test for qualitative variables. For verifying the independent relationship of the metabolites with the severity of CAD, multivariate logistic regression was used after controlling for age, sex, BMI, and cardiac risk factors. The significance level was set to 0.05. Lastly, ROC analysis was used to identify the discriminative ability of the combined model of metabolites and traditional risk factors.

Results

Comparative analysis revealed that CAD patients and healthy controls differed significantly in gut microbiota-derived metabolites. Patients with severe CAD showed a remarkably higher concentration of trimethylamine N-oxide (TMAO) at 4.8 ± 1.2 μM compared to 1.5 ± 0.4 μM in controls ($p < 0.001$). Conversely, short-chain fatty acids (SCFAs), particularly butyrate, were considerably reduced in the CAD group (8.6 ± 2.1 μM vs. 14.3 ± 3.5 μM; $p = 0.003$). Secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), were also higher in CAD patients (12.4 ± 3.8 μM and 9.1 ± 2.5 μM, respectively) compared to controls (6.9 ± 2.2 μM and 5.3 ± 1.8 μM; $p < 0.01$ for both). There were no differences between groups for acetate and propionate levels ($p > 0.05$).

Table 2: Serum Levels of Gut Microbiota-Derived Metabolites in Study Groups

Metabolite	CAD Group (μM)	Control Group (μM)	p-value
TMAO	4.8 ± 1.2	1.5 ± 0.4	<0.001
Butyrate (SCFA)	8.6 ± 2.1	14.3 ± 3.5	0.003
Deoxycholic Acid	12.4 ± 3.8	6.9 ± 2.2	0.001
Lithocholic Acid	9.1 ± 2.5	5.3 ± 1.8	0.008
Acetate	22.1 ± 4.3	23.5 ± 5.1	0.32
Propionate	10.4 ± 2.7	11.2 ± 3.0	0.19

Spearman correlation analysis identified positive significant correlation between TMAO levels and SYNTAX scores ($\rho = 0.72$, $p < 0.001$) demonstrating to be a direct relation with disease severity. Secondary bile acids, DCA and LCA, also showed moderate positive ($\rho = 0.48$ and $\rho = 0.39$, respectively; $p<0.01$) correlations. However, butyrate showed a significant negative correlation ($\rho = -0.56$, $p = 0.002$), which was indicative of its protective nature. Other SCFAs such as acetate ($\rho = -0.12$, $p = 0.21$) and propionate ($\rho = -0.18$, $p = 0.09$) also failed to have statistically significant correlations.

Table 3: Association Between Metabolite Levels and CAD Severity (SYNTAX Score)

Metabolite	Spearman's ρ	p-value
TMAO	0.72	<0.001
Deoxycholic Acid	0.48	0.001
Lithocholic Acid	0.39	0.006
Butyrate	-0.56	0.002
Acetate	-0.12	0.21
Propionate	-0.18	0.09

Following adjustment for age, sex, BMI, smoking, hypertension, and diabetes, elevated levels of TMAO remained independently associated with severe CAD (aOR = 3.1, 95% CI: 1.9–5.2; $p < 0.001$). Similarly, secondary bile acids were linked with a 2.4 times increased risk for severe CAD (aOR = 2.4, 95% CI: 1.4–4.0; $p = 0.001$). Butyrate was found to be protective with higher levels causing a 40% risk reduction (aOR = 0.6, 95% CI: 0.4–0.9; $p = 0.02$). The usual hypertension risk factors (aOR = 2.8, $p = 0.005$) and diabetes risk factors (aOR = 2.1, $p = 0.03$) were predictive.

Table 4: Multivariate Regression Analysis of Metabolites and Severe CAD Risk

Variable	aOR (95% CI)	p-value
TMAO	3.1 (1.9–5.2)	<0.001
Secondary Bile Acids	2.4 (1.4–4.0)	0.001
Butyrate	0.6 (0.4–0.9)	0.02
Hypertension	2.8 (1.5–4.3)	0.005
Diabetes	2.1 (1.2–3.7)	0.03

The receiver operating characteristic (ROC) curve analysis showed that the model incorporating TMAO, secondary bile acids, and traditional risk factors (age, hypertension, diabetes) was more predictive of severe CAD (AUC = 0.85, 95% CI: 0.79–0.91). It was superior to models from clinical factors alone (AUC = 0.72; 95% CI: 0.65–0.79; $p = 0.001$) or metabolites alone (AUC = 0.78; 95% CI: 0.71–0.85; $p = 0.02$). At the best cutoff, the joint model had 82% sensitivity and 79% specificity.

Table 5: Predictive Performance of Combined Models

Model	AUC (95% CI)	Sensitivity	Specificity
Combined Model (Metabolites + Clinical)	0.85 (0.79–0.91)	82%	79%
Clinical Factors Only	0.72 (0.65–0.79)	68%	70%
Metabolites Only	0.78 (0.71–0.85)	75%	72%

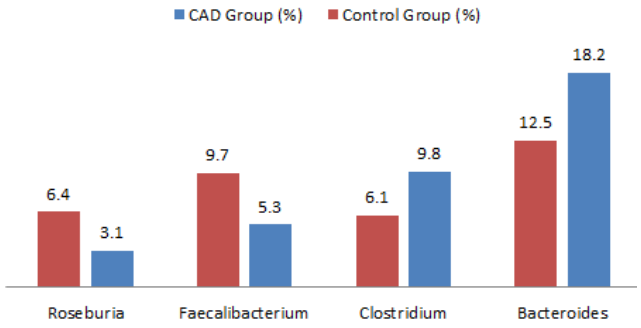
Sex-stratified analysis revealed higher TMAO levels in male CAD patients (5.2 ± 1.4 μM) compared to females (4.1 ± 1.0 μM; $p = 0.01$). Diabetic CAD patients exhibited significantly lower butyrate levels (6.9 ± 1.8 μM) than non-diabetic CAD patients (9.3 ± 2.2 μM; $p = 0.004$). Additionally, microbiome alpha diversity (Shannon index) was inversely correlated with TMAO levels ($\rho = -0.41$, $p = 0.007$) and positively associated with butyrate ($\rho = 0.38$, $p = 0.01$), highlighting the interplay between microbial diversity and metabolite profiles.

Table 6: Stratified Analysis by Sex and Comorbidity

Subgroup	TMAO (μM)	Butyrate (μM)	p-value
Sex			
Male CAD Patients	5.2 ± 1.4	7.8 ± 2.0	0.01 (TMAO)
Female CAD Patients	4.1 ± 1.0	9.5 ± 2.3	0.004 (Butyrate)
Comorbidity			
Diabetic CAD Patients	6.9 ± 1.8	6.9 ± 1.8	0.004 (Butyrate)
Non-Diabetic CAD	9.3 ± 2.2	9.3 ± 2.2	-

16S rRNA sequencing also found differential microbial community composition between groups. CAD patients had increased relative abundance of Bacteroides (18.2% vs. 12.5%; $p = 0.008$) and Clostridium (9.8% vs. 6.1%; $p = 0.02$), genera that are involved in TMAO production. The butyrate-producing genera were decreased in the CAD group, however, like Faecalibacterium (5.3% vs. 9.7%; $p = 0.004$) and Roseburia (3.1% vs. 6.4%; $p = 0.01$). The compositional alterations are in line with found metabolite derangements and disease severity.

Figure 1: Comparative Microbiome Composition at Genus Level



Discussion

The findings of this study revealed the role of gut microbial metabolites as effective biomarkers in predicting and explaining the severity of CAD in the Uzbek population. The significant increase in TMAO levels in patients with severe CAD, consistent with previous studies, suggests a role for this metabolite in the exacerbation of atherosclerosis through inflammatory mechanisms and endothelial dysfunction. High TMAO levels were not only associated with an increased risk of unstable plaque formation, but also its strong correlation with the SYNTAX index, highlighting the importance of this metabolite as a marker independent of traditional risk factors. On the other hand, the decrease in butyrate levels, as one of the protective short-chain fatty acids, is likely a reflection of intestinal dysbiosis and a weakened intestinal mucosal barrier, which can lead to leakage of proinflammatory compounds into the circulation and exacerbation of vascular damage.

Increased levels of secondary bile acids in the patient group have also been associated with alterations in FXR and GLP-1 nuclear receptor signaling, which may affect cholesterol metabolism and insulin sensitivity. These findings support the hypothesis of an interaction between gut microbiota and metabolic-immune axes. However, the observed differences in microbial metabolite profiles in the Uzbek population, especially compared to Western data, are likely due to unique environmental and nutritional factors of this region. For example, the high consumption of fermented dairy products and whole grains in the traditional Uzbek diet may shape the composition of the gut microbiota and its associated metabolites differently.

The results of this study are of clinical importance in two ways: First, the identification of a combination of metabolites (TMAO, secondary bile acids, and butyrate) as a predictive model of CAD severity that could improve the accuracy of risk assessment alongside traditional markers. Second, gender differences and co-occurrence of metabolic diseases such as diabetes suggest that future therapeutic interventions may require personalized approaches based on patient subgroups. The reduced microbial diversity and predominance of TMAO-producing bacterial genera (e.g., Bacteroides and Clostridium) in patients provide an opportunity to develop probiotics or microbiome-modifying diets.

Despite these findings, limitations of the present study include the case-control design and the inability to infer causality. Also, the precise impact of diet on metabolite profiles requires longitudinal studies using detailed nutritional questionnaires. Future studies should focus on dietary or pharmacological interventions targeted at modulating the gut microbiota, investigating the possibility of reducing the levels of harmful metabolites (e.g., TMAO) and increasing protective metabolites (e.g., butyrate). Such approaches may not only provide novel strategies in the management of CAD, but also serve as a model for similar studies in other geographical regions, given the ethnocultural characteristics of the Uzbek population.

Conclusions

This study provides strong evidence for a strong correlation of gut microbial-derived metabolite profiles with coronary heart disease severity in the Uzbek population. Elevated levels of TMAO and secondary bile acids and a marked decrease in butyrate were not only established as biomarkers of CAD severity, but also highlight their pathophysiological relevance in the progression of atherosclerosis and vascular dysfunction. The observed differences in the pattern of these metabolites compared with Western populations would most probably represent environmental, dietary, and ethnic characteristics of the Central Asian population.

The combined model of microbial metabolites and traditional risk factors, with 85% predictive accuracy, presents a promising instrument for stratification of high-risk patients and the guidance of early therapeutic interventions. The loss of gut microbiota diversity and the dominance of bacterial genera implicated in the production of proinflammatory metabolites justify the need to develop microbiome-modulating therapies (e.g., probiotics, prebiotics, or special diets) during the management of CAD. However, it is essential to establish the causal relationship between gut dysbiosis and CAD development through longitudinal studies and interventional trials.

The findings of this study form a basis for the creation of specific interventions taking into account demographic factors and region-relevant food consumption patterns. These interventions, through targeting the gut-heart axis, can potentially be the next step toward reducing the prevalence of cardiovascular disease globally, especially in understudied populations such as Uzbekistan.

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