BRITISH JOURNAL OF HOSPITAL MEDICINE

ARTICLE

Association between Intestinal Flora Metabolites and Coronary Artery Vulnerable Plaque Characteristics in Coronary Heart Disease

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Abstract

Aims/Background The incidence of coronary heart disease (CHD) has been increasing annually. Patients with severe conditions may die from myocardial infarction, heart failure or malignant arrhythmia. Intestinal flora plays an important role in various metabolic processes, such as atherosclerosis, tumour formation, and inflammation. However, its direct role in promoting plaque vulnerability must be further explored and validated. Therefore, this study aims to explore the relationship between changes in intestinal flora, its metabolites in CHD patients and the vulnerability characteristics of coronary plaques.

Methods This study recruited 180 subjects, among these, 90 CHD patients diagnosed between January 2023 and January 2024 were selected as the CHD group and 90 healthy volunteers were selected as the control group following a principle of 1:1 ratio. The differences in intestinal flora composition, metabolite levels, and blood biochemical indexes were compared between the two study groups. Based on the coronary angiography (CAG) and intravascular ultrasound (IVUS) results, the CHD group was divided into two sub-groups for stratified comparative analysis: the stable plaque group (n = 49) and the vulnerable plaque group (n = 41).

Results The CHD group had reduced intestinal *Bifidobacteria* and lactic acid bacteria counts and higher intestinal *Escherichia coli* and *Enterococcus* levels than the control group ($p < 0.05$). Moreover, trimethylamine-N-oxide (TMAO) and phenylacetylglutamine (PAGln) levels were significantly higher in the CHD group compared to the control group ($p < 0.05$). Similarly, the CHD group exhibited substantially elevated serum triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels compared to the control group. However, compared to the control group, the high-density lipoprotein cholesterol (HDL-C) levels were significantly lower in the CHD group ($p < 0.05$). Furthermore, the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum urea nitrogen (BUN), and serum creatinine (Scr) were comparable in the two experimental groups ($p > 0.05$). Similarly, intestinal *Bifidobacteria*, lactic acid bacteria, *Escherichia coli*, and *Enterococcus* compositions were comparable in CHD patients with vulnerable plaque and those with stable plaque ($p > 0.05$). Moreover, CHD patients with vulnerable plaque had elevated TMAO and PAGln levels than those with stable plaque ($p < 0.05$). However, TG, TC, HDL-C, LDL-C, ALT, AST, BUN, and Scr levels were comparable between CHD patients with a vulnerable plaque and those with stable plaque ($p > 0.05$). Multivariate regression analysis showed that diabetes, elevated TMAO levels, and elevated PAGln levels were potential risk factors for coronary plaque vulnerability ($p < 0.05$).

Conclusion In summary, CHD patients exhibit significant intestinal flora imbalance, with elevated TMAO and PAGln metabolite levels, which are related to the characteristics of plaque instability.

Key words: coronary disease; intestinal flora; metabolome; atheromatous plaque; fibroatheromatous plaque

Submitted: 17 October 2024 Revised: 2 December 2024 Accepted: 12 December 2024

How to cite this article:
You X, Gao B. Association between Intestinal Flora Metabolites and Coronary Artery Vulnerable Plaque Characteristics in Coronary Heart Disease. Br J Hosp Med. 2025. https://doi.org/10.12968/hmed.2024.0772

Introduction

Coronary heart disease (CHD) is an ischemic heart disease caused by atherosclerosis. Its incidence and mortality rates have been increasing annually among younger populations. In China alone, this disease continues to rise, with an estimated 290 million individuals currently affected, including 11 million coronary heart disease (CHD) patients (Sayols-Baixeras et al, 2023; Trøseid et al, 2020). It is worth noting that many CHD patients experience no specific symptoms before the acute onset of the disease, which are often triggered by the rupture of atherosclerotic plaques, especially vulnerable plaques in the coronary arteries, resulting in thrombosis (Rahman et al, 2022; Zhao et al, 2023).

Although achievements have been made in clinical treatments, research on coronary plaque vulnerability remains insufficient (Romano et al, 2023). Intravascular ultrasound (IVUS) is the primary non-invasive method for evaluating atherosclerotic plaque properties in clinical settings (Wen et al, 2022). Moreover, the role of intestinal flora in multiple metabolic processes such as atherosclerosis, tumours, and inflammation is well-established. However, its direct role in promoting the progression of plaques toward a vulnerable state needs further investigation (Zhen et al, 2023). Gut-derived metabolites such as trimethylamine-N-oxide (TMAO) and phenylacetylglutamine (PAGln) are strongly associated with cardiovascular disease risk and plaque instability. TMAO, produced by gut bacteria, is closely linked to the development of cardiovascular disease. PAGln is a metabolite produced by the gut microbiota by converting dietary phenylalanine into phenylacetic acid, which is then combined with glutamine. These metabolites contribute to the stability of coronary artery plaques through vascular dysfunction and inflammatory response. Despite their well-established role in cardiovascular disease, the association between these metabolites and the vulnerability of coronary artery plaques remains uninvestigated, with few studies preliminary addressing this relationship (Chen et al, 2023; Song et al, 2024).

Addressing this research gap, this study explored the differences in intestinal flora composition, metabolite profiles, and blood biochemical indicators between CHD patients and healthy individuals. It further assessed the correlation between these differences and coronary plaque vulnerability characteristics. Combining clinical detection techniques such as IVUS, this study offers a comprehensive analysis of the role of gut microbiota and its metabolites in CHD development. By addressing existing gaps, the study aims to fill the gap in current research on the relationship between gut microbiota and coronary plaque vulnerability and to provide a comprehensive basis for preventing, diagnosing, and treating CHD in clinical practice.

Methods

Study Participants and their Baseline Characteristics

This study recruited 90 CHD patients diagnosed at Zhejiang Veteran Hospital between January 2023 and January 2024, and healthy volunteers (n = 90) were selected as the control group following a 1:1 matching principle. The sample size

for this case-control study was computed as follows: $N = Z^2 \times \sigma^2 \div d^2$, where Z is the confidence interval, σ is the standard deviation (usually set at 0.5), and d is the sampling error range, and, N is the required sample size. CHD patients were diagnosed following the criteria established by the Cardiology Branch of the Chinese Medical Association (Ministry of Health of the People's Republic of China, 2010).

Inclusion criteria for patient recruitment were as follows: (1) Aged 30 to 70 years old, with good compliance, agreed to participate in this study, and signed informed consent. (2) Complete intestinal flora and metabolite assessments. (3) Diagnosis confirmed through coronary angiography and IVUS. (4) No use of antibiotics, non-steroidal anti-inflammatory drugs, glucocorticoids, lipid-lowering drugs, or hepatoprotective drugs within one before hospitalization. (5) The control group included healthy volunteers without coronary artery disease.

Exclusion criteria included (1) individuals with significant organ dysfunction (like liver and kidney), acute infection, malignant tumours, strokes, muscle or skeletal system diseases, chronic wasting diseases, hepatobiliary diseases, autoimmune diseases, or other severe medical and surgical conditions, (2) those with history of mental illness, (3) alcoholics, (4) individuals who participated in other clinical trials within the last 3 months, and (5) patients had received coronary artery bypass grafting.

This study design followed the principles outlined in the Declaration of Helsinki and obtained approval from the Ethics Committee of Zhejiang Veteran Hospital (No. 57 of the Ethical Approval No. 2024). Similarly, written informed consent was obtained from each study participant.

IVUS Examination and Plaque Stability Judgment Method

Initially, the target vessel was identified through routine coronary angiography (CAG) using the German Siemens Angiostar angiography. Based on the CAG results, the vessels with suspected severe lesions were further examined using an intravascular ultrasound instrument (S5, 807300-001, VOLCANO, Round Rock, TX, USA). A 20 MHz electronic polycrystalline phased-array high-frequency ultrasound catheter (Eagle Eye Platinum, 20193061627, VOLCANO, Round Rock, TX, USA) with a diameter of 2.9 F was used, operating with an automatic withdrawal speed of 0.5 mm/s. The short-axis and sagittal ultrasound images of the distal, lesion, and proximal segments of the target lesion were captured.

Through repeated observation and contrast agent injection, the infiltration of the contrast agent within the plaque was evaluated to determine the presence of plaque rupture. The collected data were automatically calibrated by the IVUS console and software, which distinguished four main plaque components: green (fibrous tissue), yellow-green (fibrous adipose tissue), red (necrotic core), and white (calcified tissue).

Based on the characteristics of vulnerable plaques, such as thin fibrous cap, plaque load $>50\%$, necrotic core $>10\%$, a positive remodelling, calcified nodules, ruptures, dissections or thrombus, the Medvision-2.06 ultrasound analysis workstation was used for offline measurement and analysis. Two associate chief physicians

or senior interventional physicians assessed the results using a double-blind method, reaching a consensus.

Laboratory Index Detection Methods

Detection of TMAO and PAG1n Levels

A blood sample (3 mL) was collected from each study subject using serum vacuum collection tubes followed by centrifugation at 800 ×g for 10 minutes to obtain serum. TMAO levels were detected using an LC-30A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) and SCIEX 5600 + mass spectrometer (Shimadzu, Kyoto, Japan). However, PAGln levels were determined by employing an enzyme-linked immunosorbent assay.

Detection of Intestinal Flora

Faecal samples (>10 g) were collected following the standard procedure, labelled, and stored at –80 °C. Total genomic DNA was extracted utilizing a DNA extraction kit (MagMAX, 2402090, Thermo Fisher Scientific, Waltham, MA, USA). Following DNA purity and quality assessment, PCR amplification was performed using specific primers targeting the V4 region of the bacterial 16S rRNA gene. The amplified products were purified through gel electrophoresis to remove impurities. The microbial DNA was extracted by magnetic bead method, and the V4 region was amplified by secondary PCR. Then, 16S rRNA gene sequencing technology was used to analyze the intestinal flora. High-throughput sequencing was performed on the Illumina Novaseq 6000 platform to assess the intestinal flora structure. Based on the sequencing data, the intestinal flora composition was calculated, and differences between groups were compared and analyzed.

Blood Lipid Test

After fasting for at least 8 hours, 3 mL of blood sample was collected in the morning without anticoagulation. After centrifugation (1000 ×g, 10 min), serum was obtained for subsequent analysis. Levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were detected utilizing an automatic biochemical analyzer (CX9, Beckman Coulter Inc, Brea, CA, USA).

Liver and Kidney Function Testing

A fasting venous blood sample (3 mL) was collected from each patient with anticoagulation. After centrifugation (3000 rpm, 10 min), the supernatant was used for subsequent analysis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum urea nitrogen (BUN), and serum creatinine (Scr) levels were determined using a Beckman CX9 automatic biochemical analyzer.

Statistical Analysis

Statistical analysis was conducted using SPSS21.0 (IBM, Armonk, NY, USA). Normality in data was assessed using the Shapiro-Wilk test. The collected data on intestinal flora, TMAO, and PAGln expression were categorized as measurement data. The number of intestinal flora was normalized through logarithmic transfor-

mation, while the remaining measurement indicators, following the requirements of normal distribution, were expressed as the mean ± deviation ($\bar{x} \pm s$). The *t*-test was used for comparison between the two groups.

Categorical data, including gender distribution, lifestyle factors, hypertension rate, and diabetes prevalence, were expressed as percentages (%) and statically compared using the χ^2 test. Multivariate analysis was performed using a logistic regression model. A *p*-value of <0.05 was considered statistically significant.

Results

Comparison of Baseline Characteristics between the CHD and Control Groups

The baseline characteristics, such as age, body mass index (BMI), gender distribution, lifestyle factors, hypertension rate, and diabetes prevalence, demonstrated no significant differences between the CHD and control groups ($p > 0.05$, Table 1).

Comparison of Intestinal Flora, TMAO, and PAGln between the CHD and Control Groups

The number of intestinal *Bifidobacteria* and lactic acid bacteria was lower in the CHD group than in the control group. Conversely, the intestinal *Escherichia coli* and *Enterococcus* counts were higher in the CHD group than in the control group ($p < 0.05$). Furthermore, serum TMAO and PAGln levels were significantly higher in the CHD group compared to the control group ($p < 0.05$) (Table 2).

Comparison of Biochemical Indexes between the Two Experimental Groups

Serum TG, TC, and LDL-C levels were significantly elevated in the CHD group compared to the control group. However, HDL-C levels were substantially lower in the CHD group than in the control group ($p < 0.05$). Additionally, ALT, AST, BUN, and Scr levels were comparable between the CHD and control groups ($p > 0.05$) (Table 3).

Comparison of Intestinal Flora, TMAO and PAGln between CHD Group and Control Group with Different Plaque Characteristics

The number of intestinal *Bifidobacteria*, lactic acid bacteria, *Escherichia coli* and *Enterococcus* in CHD patients with vulnerable plaque was compared with that in the stable plaque group ($p > 0.05$). The serum levels of TMAO and PAGln in CHD patients with a vulnerable plaque were higher than those in the stable plaque group ($p < 0.05$) (Table 4).

Comparison of Biochemical Indexes between the CHD and Control Groups with Different Plaque Characteristics

The serum levels of TG, TC, HDL-C, LDL-C, ALT, AST, BUN, and Scr in CHD patients with vulnerable plaque were comparable to those in the stable plaque group ($p > 0.05$, Table 5).

Table 1. Comparison of baseline characteristics between the CHD and control groups.

Experimental group	n	Age (years old)	BMI (kg/m^2)	Gender (%)		Smoking history (%)	Drinking history (%)	Hypertension (%)	Diabetes (%)
				Male	Female				
CHD group	90	61.31 ± 6.81	23.77 ± 2.16	56 (62.22)	34 (37.78)	35 (38.89)	30 (33.33)	21 (23.33)	28 (31.11)
Control group	90	62.07 ± 6.43	23.54 ± 2.28	50 (55.56)	40 (44.44)	30 (33.33)	36 (40.00)	27 (30.00)	33 (36.67)
t/χ^2		–0.770	0.695	0.826		0.602	0.861	1.023	0.620
p-value		0.442	0.488	0.363		0.438	0.353	0.312	0.431

Note: CHD, coronary heart disease; BMI, body mass index.

Table 2. Comparison of intestinal flora, TMAO, and PAGln between the CHD and control groups ($\bar{x} \pm s$).

Experimental group	n	*Bifidobacterium dentium* (IgCFU/g)	Lactic acid bacteria (IgCFU/g)	*Colibacillus* (IgCFU/g)	*Enterococcus* (IgCFU/g)	TMAO (ng/mL)	PAGln (μmol/L)
CHD group	90	6.84 ± 1.40	5.96 ± 1.20	7.43 ± 1.80	6.90 ± 1.30	316.9 ± 58.5	1.51 ± 0.56
Control group	90	7.70 ± 1.53	6.88 ± 1.47	6.50 ± 1.54	6.22 ± 1.28	251.7 ± 53.9	0.98 ± 0.34
t		–3.934	–4.599	3.724	3.536	7.776	7.675
p-value		<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Note: TMAO, trimethylamine-N-oxide; PAGln, phenylacetylglutamine.

Table 3. Comparison of biochemical indexes between the CHD and control groups ($\bar{x} \pm s$).

Experimental group	n	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ALT (U/L)	AST (U/L)	BUN (mmol/L)	Scr (μmol/L)
CHD group	90	2.11 ± 0.40	6.51 ± 0.98	0.92 ± 0.12	3.58 ± 0.70	24.61 ± 5.80	26.03 ± 6.50	7.02 ± 1.80	93.41 ± 9.63
Control group	90	1.80 ± 0.44	5.92 ± 0.93	1.03 ± 0.15	3.17 ± 0.68	23.60 ± 5.41	24.55 ± 6.92	7.21 ± 1.90	95.18 ± 10.77
t		4.946	4.143	–5.433	3.986	1.208	1.479	–0.689	–1.162
p-value		<0.001	<0.001	<0.001	<0.001	0.229	0.141	0.492	0.247

Note: TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, serum urea nitrogen; Scr, serum creatinine.

Table 4. Comparison of intestinal flora, TMAO and PAGln between the CHD and control groups with different plaque characteristics ($\bar{x} \pm s$).

Plaque characteristics	n	*Bifidobacterium dentium* (IgCFU/g)	Lactic acid bacteria (IgCFU/g)	*Colibacillus* (IgCFU/g)	*Enterococcus* (IgCFU/g)	TMAO (ng/mL)	PAG1n (μmol/L)
Vulnerable plaque	41	6.70 ± 1.20	5.73 ± 1.13	7.69 ± 1.67	7.10 ± 1.20	335.8 ± 55.7	1.63 ± 0.52
Stable plaque	49	6.96 ± 1.27	6.15 ± 1.09	7.21 ± 1.60	6.73 ± 1.14	301.1 ± 48.5	1.40 ± 0.48
t		–0.992	–1.790	1.389	1.497	3.159	2.180
p-value		0.324	0.077	0.168	0.138	0.002	0.032

Note: TMAO, trimethylamine-N-oxide; PAGln, phenylacetylglutamine.

Table 5. Comparison of biochemical indexes between the CHD patients with vulnerable plaque and individuals with stable plaque ($\bar{x} \pm s$).

Plaque characteristics	n	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ALT (U/L)	AST (U/L)	BUN (mmol/L)	Scr (μmol/L)
Vulnerable plaque	41	2.05 ± 0.35	6.65 ± 0.92	0.91 ± 0.10	3.67 ± 0.65	25.80 ± 5.53	24.94 ± 6.27	7.16 ± 1.71	95.02 ± 8.96
Stable plaque	49	2.16 ± 0.38	6.39 ± 0.95	0.93 ± 0.11	3.50 ± 0.54	23.61 ± 5.11	26.94 ± 5.80	6.90 ± 1.64	92.06 ± 9.80
t		–1.417	1.312	–0.895	1.356	1.950	–1.570	0.735	1.483
p-value		0.160	0.193	0.373	0.179	0.054	0.120	0.465	0.142

Note: TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, serum urea nitrogen; Scr, serum creatinine.

Table 6. Multivariate analysis of the vulnerability of coronary artery plaques.

Variable	β	SE	Walds	p-value	OR	95% CI	
Diabetes	0.518	0.246	4.434	0.045	1.679	1.036	2.719
TMAO	0.610	0.298	4.190	0.047	1.840	1.026	3.301
PAG1n	0.541	0.237	5.211	0.024	1.718	1.079	2.733
Constant term	1.390	0.583	5.685	0.011	4.015	1.281	12.587

Note: TMAO, trimethylamine-N-oxide; PAGln, phenylacetylglutamine; SE, standard error; OR, odds ratio; CI, confidence interval.

Multivariate Analysis of the Vulnerability of Coronary Artery Plaques

A logistic regression model was established using the statistically significant single-factor (univariate) analysis, including diabetes, TMAO, and PAGln as independent variables and the vulnerable coronary plaque as the dependent variables. The results showed that diabetes, elevated TMAO levels, and elevated PAGln levels were potential risk factors for coronary plaque vulnerability ($p < 0.05$, Table 6).

Discussion

The vulnerability of coronary atherosclerotic plaques is a crucial factor affecting the prognosis of patients with coronary heart disease (Zheng et al, 2023). This study focused on the vulnerability characteristics of plaque as a starting point to compare differences in intestinal flora composition, metabolite levels, and blood biochemical indicators between patients with coronary heart disease and healthy individuals. Furthermore, the study analyzed the relationship between these biomarkers and coronary artery plaque vulnerability. The purpose is to provide a basis for formulating and optimizing treatment strategies for coronary heart disease.

Analysis of the differences in intestinal flora between the CHD and control groups demonstrated that the CHD group had significantly lower intestinal *Bifidobacterium* and *lactobacillus* and higher *Escherichia coli* and *Enterococcus* compared to the control group. These differences were statistically significant and suggest that changes in the bacterial populations correlate with CHD. As a unique ecosystem within the body, the intestinal flora plays a crucial role in maintaining overall health (Amrein et al, 2020). In recent years, it has been increasingly recognized that the imbalance of gut flora is closely linked to chronic inflammatory conditions. This imbalance can contribute to the occurrence and development of atherosclerosis in multiple mechanisms. Previous studies have revealed that probiotics are vital in regulating inflammatory responses, optimizing cholesterol metabolism, and maintaining intestinal barrier function (Alpert, 2023; Dong et al, 2023). A reduction in probiotic populations is linked to an increased risk of cardiovascular disease. The results of this study align with these findings, indicating that *Bifidobacterium* and lactic acid bacteria, as beneficial gut bacteria, enhance immune function and produce metabolites such as short-chain fatty acids that support cardiovascular health, thereby preventing the risk of coronary heart disease. On the contrary, the number of potential pathogenic bacteria like *Escherichia coli*

and *Enterococcus* was significantly higher in CHD patients. This imbalance in gut microbiota may increase inflammation and oxidative stress, promoting plaque instability. While previous studies have revealed a correlation between gut microbiota imbalance and cardiovascular disease, this study proves further validation through a detailed analysis of changes in specific microbiota.

This study further analyzed the relationship between changes in intestinal flora metabolites and the occurrence of CHD. The findings showed that serum levels of TMAO and PAGln in CHD patients were significantly higher than those in the control group, suggesting that increased levels of these metabolites were associated with the development of CHD. Increased TMAO levels disrupt endothelial cell junction proteins and aggravate vascular inflammation and oxidative stress, thereby inducing endothelial cell dysfunction. Additionally, TMAO up-regulates scavenger receptors, enhances macrophage recognition and uptake of oxidized low-density lipoprotein, promotes foam cell formation, and thus promotes the progression of CHD (Konieczny and Kuliczkowski, 2022).

PAGln, a metabolite produced by the intestinal flora after converting dietary phenylalanine into phenylacetic acid and subsequently combining it with glutamine, transmits signals through adrenergic receptors, affecting physiological functions. Previously, it has been shown that PAGln levels are associated with cardiovascular disease risk and serve as an independent cardiovascular risk factor (Zhu et al, 2023). In our study, the increased serum PAGln levels in CHD patients may indicate the accumulation of harmful metabolites resulting from gut microbiota imbalances, especially the increase in potential pathogenic bacteria. These findings suggest the role of intestinal flora metabolites in the pathogenesis of CHD and provide a direction for clinical intervention. Regulating gut microbiota and its metabolites may provide a viable approach in preventing and treating CHD.

Furthermore, no significant difference was found in ALT, AST, BUN, and Scr levels between the CHD and control groups, suggesting that liver and kidney function were not the primary impacting factors for CHD. However, the blood lipid analysis showed that serum TG, TC, and LDL-C levels were significantly increased in the CHD group, while HDL-C levels were decreased. These findings suggest a close association between dyslipidemia and the occurrence of CHD. High TG, TC, and LDL-C levels promote lipid deposition in the vascular walls, facilitating plaque formation, vascular stenosis, and impaired blood flow (Shaya et al, 2022). In contrast, HDL-C exhibits an anti-atherosclerotic effect by promoting reverse cholesterol transport to the liver for metabolism, thereby reducing lipid deposition. A decrease in HDL-C levels weakens this effect, providing favourable conditions for the development of CHD.

CAG is the standard method for diagnosing CHD, providing an evaluation of lumen stenosis and plaque counts. However, it is difficult to accurately identify vulnerable plaques. IVUS technology, which combines ultrasound and catheter technology, offers a more comprehensive assessment of vascular wall characteristics and plaque vulnerability. The role of intestinal flora and its metabolites in various stages of coronary heart disease has been well-documented, with significant changes observed as the disease progresses (Liu et al, 2020). Additionally, this

study examined the relationship between intestinal flora metabolites and plaque properties. We observed that serum TMAO and PAGln levels were higher in patients with vulnerable plaque than those with stable plaque. This suggests a relationship between increased TMAO and PAGln levels and plaque vulnerability in CHD. Recently, Zhang et al (2023) reported that TMAO, a product of intestinal microbiota metabolism of choline and L-carnitine, significantly increases the risk of cardiovascular disease by promoting oxidative stress, inflammatory response and endothelial dysfunction. Consistent with these results, this study shows that increased TMAO levels can destabilize plaque and predispose them to rupture by affecting bile metabolism, activating platelets and inflammatory cells, and exacerbating oxidative stress and inflammatory responses. Although the specific mechanism of PAGln remains unclear, our study indicates it may interfere with lipid metabolism through similar pathways, promote foam cell formation, and facilitate lipid deposition, thereby reducing plaque stability. Furthermore, intestinal flora imbalance may aggravate 'intestinal leakage', enabling bacteria and their metabolites to enter the blood system, which stimulates systemic inflammatory response and increases plaque vulnerability.

Comparison of serum TG, TC, HDL-C, LDL-C, ALT, AST, BUN, and Scr levels between CHD patients with vulnerable and stable plaques demonstrated no significant difference. This suggests that traditional blood lipids and biochemical parameters may not directly impact plaque vulnerability. Although dyslipidemia is a well-established risk factor for CHD, this study shows it does not significantly affect plaque characteristics (Du et al, 2023). Plaque vulnerability is regulated by a complex interaction of factors, such as inflammatory response, oxidative stress, and apoptosis, rather than closely by traditional biochemical indicators. Furthermore, this study innovatively underscores the role of intestinal flora and its metabolites, extending beyond conventional risk factors and providing new perspectives on plaque vulnerability. These results have clinical significance in evaluating novel diagnostic and therapeutic approaches for CHD.

However, this study has limitations which need to be addressed. Being a retrospective single-center study, this analysis is subject to selection bias and limited representativeness and universality. This study focuses on correlation analysis, emphasizing further comprehensive research to establish potential causal relationships. Moreover, the influence of genetic and regional factors on the findings was not considered, warranting more extensive research to validate these observations.

Conclusion

In summary, CHD patients exhibit significant gut microbiota dysbiosis, along with increased TMAO and PAGln metabolite levels. TMAO aggravates inflammatory reactions, oxidative stress, and endothelial dysfunction and enhances platelet activity and promotes thrombosis. PAGln interferes with lipid metabolism and promotes foam cell formation. Together, these metabolites promote the development of coronary artery plaque toward a vulnerable state. In clinical practice, treatments aimed at regulating gut microbiota and managing TMAO and PAGln levels, such as

nutrition adjustments or targeted therapies, may help reduce plaque instability and lower the risk of cardiovascular events. Further studies should focus on exploring the specific mechanisms of action of TMAO and PAGln to develop new strategies for CHD treatment.

Key Points

- CHD patients exhibit significant gut microbiota dysbiosis.
- The level of TMAO and PAGln metabolites is increased in CHD patients.
- Higher TMAO and PAGln levels contribute to developing coronary artery vulnerable plaque.
- In CHD patients, regulating the gut microbiota and managing TMAO and PAGln levels through dietary adjustments may reduce plaque instability and decrease the risk of cardiovascular events.

Availability of Data and Materials

All data included in this study are available from the corresponding author upon reasonable request.

Author Contributions

XY conceived the concept of the study, performed the research and wrote the draft. BG designed the research and revised the draft. Both authors contributed to important editorial changes of important content in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhejiang Veteran Hospital (No. 57 of the Ethical Approval No. 2024). Written informed consent was obtained from each study participant.

Acknowledgement

Not applicable.

Funding

This study is supported by the Basic Research Project of Zhejiang Provincial Department of Education (Y202352139).

Conflict of Interest

The authors declare no conflict of interest.

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