

Comparisons of microbiota-generated metabolites in patients with young and elderly acute coronary syndrome

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ABSTRACT

Objective: Acute coronary syndrome (ACS) is a leading cause of death worldwide. There is great interest in defining the risk factors and underlying mechanisms of ACS among young people. The microbiota and its metabolites have recently become a popular research topic, yet there is still no study that investigated microbiota-generated metabolites as a possible risk factor in young patients with ACS. In this study, we aimed to investigate the relationship between microbiota-generated metabolites and ACS in young people.

Methods: This study included 44 young patients with ACS (<50 years of age), 39 elderly patients with ACS, and 44 patients with normal coronary arteries. Inflammatory parameters and serum trimethylamine N-oxide (TMAO) and choline levels were measured in all patients.

Results: Young patients with ACS had significantly higher levels of TMAO and choline compared to the control and elderly ACS groups. Also, elderly patients with ACS had a significantly higher level of TMAO than the control group. Linear regression analysis was performed to determine the independent predictors of TMAO. Two regression models were involved. The first model included young ACS and control groups, while the second model included young and elderly ACS groups. In the first model, we found that young ACS ($\beta=0.399$, $p=0.004$) and smoking ACS ($\beta=0.211$, $p=0.046$) were significantly associated with TMAO level. In the second model, young ACS was significantly associated with TMAO level ($\beta=0.230$, $p=0.035$).

Conclusion: In this study, we have shown that young ACS was significantly associated with increased TMAO level. (*Anatol J Cardiol* 2020; 24: 175-82)

Keywords: acute coronary syndrome, young, microbiota, trimethylamine N-oxide

Introduction

Coronary artery disease (CAD) is now the leading cause of death worldwide, and its incidence is increasing (1). The etiology of CAD is multifactorial, including many traditional and non-traditional risk factors (2, 3). Age is one of the most important traditional risk factors for CAD (4). Although the possibility of CAD increases with age, its incidence has recently increased among young people (5). Defining the risk factors and underlying mechanisms of CAD in young people is of great interest. Identifying and modifying risk factors may possibly prevent and/or slow the progression of the disease.

Recently, novel nontraditional risk factors have been reported to explain the pathogenesis of CAD, including procoagulant, homocysteine, and inflammatory markers (2, 3). Microorganisms

found on the human body are defined as the “microbiota,” which has also become popular as a possible novel nontraditional risk factor for CAD. These microbiota metabolize overconsumed proteins and metabolites such as cholines and phosphatidylcholines which generate trimethylamine (TMA). TMA is rapidly absorbed and is converted to trimethylamine N-oxide (TMAO) in the liver (6). Increased serum TMAO has been shown to increase the risk of atherosclerosis by increasing proinflammatory cytokines and thrombus formation. Moreover, increased serum TMAO has been found to be associated with adverse cardiac events (6, 7).

To date, no study has investigated microbiota-generated metabolites in young patients with acute coronary syndrome (ACS) as a risk factor for CAD development. We hypothesized that increased microbiota-generated metabolites may be a risk factor for CAD in young patients and therefore investigated the TMAO level in young patients with ACS.

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Methods

Study population

This study was designed as a cross-sectional case-control study comprised of 44 young patients with ACS [< 50 years old (8)] and 39 elderly patients with ACS who underwent coronary angiography (cases) and 44 patients who underwent angiography and were found to have normal coronary arteries (controls).

Patients who did not have a final diagnosis of atherosclerotic ACS after coronary angiography ($n=6$), those with a previous history of CAD, malignancy, heart failure, severe heart valve disease, acute infection, rheumatic or hematological disease, liver disease, renal failure, or intestinal disease, as well as those who recently used an antimicrobial drug, were eliminated from the study. This study was approved by our Local Ethics Committee and was carried out according to the rules of the Declaration of Helsinki. Informed consent was obtained from all patients. ACS was classified as follows: unstable angina pectoris (USAP) if patients have ischemic chest discomfort exceeding 20 min, whose pain was not relieved with nitrates and had emerged within the last 48 h, with normal serum troponin levels, and with or without ST-segment deviation on electrocardiography (ECG); non-ST-elevation myocardial infarction (NSTEMI) if the patient developed a rise in cardiac biomarker levels (troponin I and creatine kinase-MB) during clinical follow-up; STEMI if there was permanent ST and J point elevation in at least 2 contiguous leads [>2 mm (0.2 mV) in men or >1.5 mm (0.15 mV) in women] in leads V2–V3 and/or of >1 mm (0.1 mV) in other contiguous chest or limb leads (9-11).

Coronary angiography

Coronary angiography was performed using the standard Judkins technique via femoral or radial access. Two experienced cardiologists who were blinded to the patient's data evaluated all digital angiographic images. The presence of 50% stenosis in the left main coronary artery and 70% stenosis in other major coronary arteries was considered as critical stenosis. The number of vessels with critical stenosis is also recorded.

Biochemical analysis and microbiota parameters

Blood samples were taken from all patients on admission. These blood samples were centrifuged for 10 min at 3000 rpm, and the serum was stored at -80°C in aliquots until the day of analysis. The samples were analyzed using standard laboratory methods to determine electrolytes, blood glucose, total cholesterol, low-density lipoprotein, high-density lipoprotein (HDL), and triglyceride levels. Serum TMAO and choline levels were measured using enzyme-linked immunosorbent assays (Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China) according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software (version 22.0; IBM Corp., Armonk, NY, USA).

The Kolmogorov–Smirnov test was used to test the normal distribution of the data. Continuous variables were expressed as the mean \pm standard deviation (SD) and median (first and third quartiles) according to the distribution. The Kruskal–Wallis test was used for three-group comparisons, and the Mann–Whitney U test was used for two-group comparisons. The chi-square test was used to compare categorical variables. The Spearman correlation coefficient was used to determine the correlation of TMAO with other continuous variables. We performed linear regression analysis to determine the significant predictors of TMAO, and a two-tiered approach was used. The first stage consisted of young ACS and young control groups, whereas the second stage consisted of young ACS and elderly ACS groups. A p -value <0.05 was considered statistically significant.

Results

Over-all, 44 young patients with ACS, 39 elderly patients with ACS, and 44 patients with normal coronary arteries were included in this study. The baseline characteristics of the study population are presented in Table 1. There was no significant difference among the groups in terms of baseline characteristics except age ($p<0.001$).

The laboratory parameters of the study groups are listed in Table 2. HDL cholesterol ($p=0.001$), triglycerides ($p=0.003$), ESR ($p=0.001$), and hsCRP ($p<0.001$) were significantly different among the three groups (Fig. 1).

The microbiota parameters of the study groups are shown in Table 3. Serum TMAO and choline levels were significantly different among the three groups. To determine the clinical importance of these parameters in young patients with ACS, a comparison of the three study groups was made. It was found that young patients with ACS had significantly higher levels of TMAO and choline compared to both the control and elderly ACS groups and elderly patients with ACS had significantly higher levels of TMAO than the control group (Fig. 2).

The Spearman correlation coefficient was used to determine the correlation between microbiota parameters and continuous variables. When all participants were included in the analysis (control group and ACS patients), TMAO was positively correlated with hsCRP ($r=0.311$, $p<0.001$), ESR ($r=0.324$, $p=0.006$), and BMI ($r=0.200$, $p=0.024$), while choline was positively correlated with hsCRP ($r=0.222$, $p=0.012$) and BMI ($r=0.235$, $p=0.008$). When only ACS patients were included in the analysis (young and elderly ACS patients), choline was negatively correlated with age ($r=-0.243$, $p=0.027$).

Linear regression analysis was performed to determine the statistically significant prognostic factors (significant predictors) of TMAO. Two regression models were involved. The first model included young ACS and control groups, while the second model included young ACS and elderly ACS groups. In the first model, we found that young ACS ($\beta=0.399$, $p=0.004$) and smoking

Table 1. Comparison of baseline characteristics among groups

Variables	Control group (n=44)	Young ACS (n=44)	Elderly ACS (n=39)	P	Control vs. young	Control vs. elderly	Young vs. elderly
Age, years	46 (41-55)	48 (44-50)	65 (61-70)	<0.001	0.827	<0.001	<0.001
Male gender (%)	23 (52)	30 (68)	28 (72)	0.137	0.130	0.070	0.722
BMI (kg/m ²)	26.5±3.2	27.6±2.3	27.0±2.4	0.135	0.062	0.377	0.253
DM (%)	3 (7)	4 (9)	4 (10)	0.851	0.695	0.576	0.858
HT (%)	6 (14)	11 (25)	5 (13)	0.880	0.179	0.388	0.37
Smoking (%)	8 (18)	6 (14)	6 (15)	0.841	0.562	0.736	0.822
SBP (mm Hg)	126 (120-138)	120 (110-130)	124 (120-130)	0.310	0.059	0.643	0.150
DBP (mm Hg)	75 (70-83)	70 (65-80)	73 (70-80)	0.305	0.239	0.743	0.153
LVEF (%)	62 (58-65)	55 (58-60)	55 (50-57)	0.058	0.056	0.080	0.062
Type of ACS (%)				0.556			
USAP		10 (23)	12 (31)				
NSTEMI	-	11 (25)	12 (31)				
STEMI		23 (52)	15 (39)				
Number of vessels with critical stenosis (%)				0.253			
1-vessel disease		18 (41)	10 (26)				
2-vessel disease		17 (39)	16 (41)				
3-vessel disease		9 (20)	13 (33)				
Final therapy (%)	-						
Medical therapy		0 (0)	4 (10.3)	0.029			
PCI		38 (86)	27 (62.2)	0.404			
CABG		6 (14)	8 (20.5)	0.742			

ACS - acute coronary syndrome; BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; DM - diabetes mellitus; HT- hypertension; EF- ejection fraction; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI - ST-segment elevation myocardial infarction; USAP - unstable angina pectoris; CAD - coronary artery disease; PCI - percutaneous coronary intervention; CABG - coronary artery bypass graft

ACS ($\beta=0.211$, $p=0.046$) were significantly associated with TMAO level. In the second model, young ACS was significantly associated with TMAO level ($\beta=0.230$, $p=0.035$) (Table 4).

Discussion

In this present study, we found that young patients with ACS had significantly higher levels of TMAO and choline compared to the control and elderly ACS groups. To our knowledge, this is the first study demonstrating an independent relationship between TMAO and young ACS.

Atherosclerosis is the main cause of CAD, and many traditional and nontraditional risk factors play a role in its etiology (12). Recent studies have demonstrated that other factors such as the immune system, inflammatory process, and vascular changes may play important roles in the pathogenesis of CAD (13, 14). The microbiota is also a novel nontraditional risk factor (6). Several studies have shown the relation of the microbiota and its imbalance to CAD as well as its major risk factors.

Microbiota as a potential diagnostic marker for CAD was also reported (15, 16).

There are many beneficial bacteria on various parts of the body, including the skin, mouth, and intestines. The bacteria in these regions are identified as microbiota and consist of various microorganisms in healthy individuals. The microbiota begins to develop immediately after birth and varies according to nutrition, genetics, age, and geographical region. These microorganisms play an important role in the physiological, metabolic, and immune processes of our bodies. Therefore, the microbiota has been defined as a new metabolic organ (17, 18).

The identification of new microbiota-generated metabolites and pathways associated with cardiovascular risk in humans is the most interesting evidence for the role of the intestinal microbiota in cardiovascular disease. Choline is an essential nutrient for the human body, but people cannot synthesize enough choline for their metabolic needs. Therefore, a sufficient amount of choline should be taken through diet (19, 20). However, overconsumption of choline may lead to the production of metabolites that are harmful to the human body. The most harmful dead-

Table 2. Comparison of laboratory parameters among groups

Variables	Control group (n=44)	Young ACS (n=44)	Elderly ACS (n=39)	P	Control vs. young	Control vs. elderly	Young vs. elderly
WBC (10 ⁹ /µL)	10 (9-11)	11 (10-13)	10 (8-13)	0.780	0.040	0.844	0.135
Hemoglobin (g/dL)	12.5±1.5	13.3±1.5	13.2±2.1	0.129	0.152	0.275	0.442
Platelets (10 ⁹ /µL)	264 (209-294)	228 (196-290)	234 (209-290)	0.185	0.056	0.315	0.578
Glucose (mg/dL)	88 (83-95)	91 (85-96)	85 (83-93)	0.132	0.162	0.598	0.062
Urea (mg/dL)	31.2±10.5	29.7±10.0	30.5±10.3	0.184	0.494	0.752	0.728
Creatinine (mg/dL)	0.8±0.20	0.9±0.19	0.9±0.20	0.280	0.132	0.120	0.908
Sodium (mEq/L)	138.6±2.3	138.2±4.6	139.5±4.1	0.274	0.738	0.078	0.288
Potassium (mEq/L)	4.2±0.4	4.2±0.5	4.3±0.4	0.463	0.521	0.192	0.553
AST (U/L)	19 (16-25)	19 (14-29)	19 (15-25)	0.658	0.235	0.603	0.210
ALT (U/L)	16 (13-20)	25 (19-47)	19 (14-28)	0.110	0.430	0.204	0.070
Total-C (mg/dL)	178.5±26.9	162.6±39.8	168.7±53.2	0.184	0.030	0.285	0.554
LDL-C (mg/dL)	102 (90-122)	107 (72-115)	103 (7-128)	0.819	0.499	0.767	0.834
HDL-C (mg/dL)	46 (39-56)	37 (32-42)	34 (29-45)	0.001	0.001	0.002	0.982
TG (mg/dL)	114 (76-150)	167 (121-284)	169 (107-227)	0.003	0.008	0.092	0.688
hsCRP (mg/dL)	0.58 (0.20-3.20)	6.70 (2.70-14.4)	2.44 (1.60-7.40)	<0.001	<0.001	0.509	0.019
ESR (mm/h)	6 (3-13)	18 (9-26)	23 (10-42)	0.001	0.130	<0.001	0.135

ACS - acute coronary syndrome; ESR - erythrocyte sedimentation rate; hsCRP - high-sensitivity C-reactive protein; AST - aspartate aminotransferase; ALT - alanine aminotransferase; total-C - total cholesterol; HDL - high-density lipoprotein; LDL - low-density lipoprotein; TG - triglyceride; WBC - white blood cell

Table 3. Comparison of microbiota parameters among groups

Variables	Control group (n=44)	Young ACS (n=44)	Elderly ACS (n=39)	P	Control vs. young	Control vs. elderly	Young vs. elderly
Choline (ng/mL)	0.45 (0.43-0.61)	0.62 (0.52-4.10)	0.54 (0.51-0.59)	0.001	<0.001	0.008	0.013
TMAO (µg/mL)	10 (8.60-13.31)	57 (13-123)	21 (10-95)	0.001	<0.001	<0.001	0.041

TMAO - trimethylamine N-oxide; ACS - acute coronary syndrome

end metabolite of the intestinal microbiota is TMAO, which is derived from choline. In addition to choline, choline-containing compounds such as red meat, eggs, and dairy products, as well as lecithin and carnitine, are potential precursors of TMAO. Fish and other seafood also contain an important amount of free TMAO. However, the association between increased TMAO and atherosclerosis seems like a paradox because seafoods are generally accepted as cardioprotective foods. The main reason for this paradox is that the role of free TMAO in seafood has commonly been overlooked. Free TMAO in seafood is directly absorbed from the gastrointestinal tract, and its level in plasma increases rapidly, whereas TMAO derived from choline and other precursors is produced in the liver by the oxidation of TMA by the microbiota, and its levels in plasma increase more slowly. Therefore, whether TMAO is taken up directly or as TMA after bacterial reduction is important (21). Studies have demonstrated that TMAO derived from hepatic oxidation by the microbiota is

associated with the risk of atherosclerosis (22). Landfald et al. (21) related this paradox to a red herring.

Previous studies proposed that increased serum TMAO was associated with an increased risk of atherosclerosis and adverse cardiac events (7). The main possible underlying mechanism linking increased TMAO with atherosclerosis risk has been explained as follows. First, TMAO increases pro-inflammatory cytokines but decreases anti-inflammatory cytokines. Second, it promotes lipid accumulation and foam cell formation. Third, it increases platelet activation and aggregation. Fourth, it causes endothelial cell dysfunction and vascular inflammation via oxidative stress (7, 23). Therefore, an increased TMAO level is associated with pro-atherogenic and prothrombotic events that result in ACS. Unlike in these studies, a recent study testing the association between TMAO and coronary artery calcium (CAC) and carotid intima-media thickness (cIMT) found that TMAO was not associated with measures of atherosclerosis (CAC inci-

Table 4. Multivariate linear regression analyses showing significant predictor of the TMAO

Model 1 (young ACS and control group)	Unstandardized coefficients		Standardized coefficients		
	B	SE	β	t	P
Age	0.641	0.877	0.080	0.732	0.467
Gender	19.616	14.392	0.153	1.363	0.177
BMI	4.688	2.427	0.212	1.931	0.057
HT	7.526	17.229	0.047	0.437	0.664
DM	14.469	24.692	0.062	0.586	0.560
Smoking	36.184	17.811	0.211	2.032	0.046
SBP	0.462	0.518	0.093	0.891	0.376
Creatinine	43.358	36.584	0.139	1.185	0.240
Total-C	0.148	0.220	0.082	0.674	0.502
LDL	0.188	0.242	0.094	0.776	0.440
HDL	0.036	0.470	0.009	.077	0.939
Triglyceride	0.013	0.055	0.028	0.242	0.809
hsCRP	0.260	0.550	0.054	0.473	0.637
Young ACS	50.046	16.748	0.399	2.988	0.004
Model 2 (young and elderly ACS)					
Age	2.309	1.870	0.387	1.235	0.221
Gender	4.627	15.389	0.035	0.301	0.765
BMI	0.664	3.329	0.026	0.200	0.842
HT	3.896	19.549	0.024	0.199	0.843
DM	10.991	22.386	0.053	0.491	0.625
Smoking	1.832	21.310	0.011	0.086	0.932
SBP	0.476	0.530	0.100	0.899	0.371
Creatinine	13.381	36.058	0.041	0.371	0.712
Total-C	0.362	0.210	0.272	1.727	0.088
LDL	0.383	0.221	0.274	1.736	0.087
Triglyceride	0.033	0.046	0.083	0.718	0.475
hsCRP	0.180	0.546	0.074	0.346	0.536
Young ACS	28.275	13.139	0.230	2.152	0.035

B - unstandardized regression coefficient; SE - standard error; β - standardized β coefficient; BMI - body mass index; DM - diabetes mellitus; HT - hypertension; hsCRP - high-sensitivity C-reactive protein; total-C - total cholesterol; SBP - systolic blood pressure; TMAO - trimethylamine N-oxide; LDL - low-density lipoprotein; HDL - high-density lipoprotein; ACS - acute coronary syndrome

dence, CAC progression, or cIMT) (24). The main reason for this unexpected result may be that the subjects in this study were relatively young and healthy.

In our study, young patients with ACS had significantly higher levels of TMAO and choline compared to the control and elderly ACS groups. This impressive finding may be attributed to a higher ratio of unhealthy and fast-food habits among young patients with ACS since unhealthy diets among young people have become more popular worldwide has been recognized (25). Indeed, the fact that we found a negative correlation between age and choline level supports our hypothesis that unhealthy eat-

ing habits are more common in young ACS patients. Moreover, young ACS was significantly associated with increased TMAO level. Therefore, it may be concluded that microbiota-generated metabolites might be causally related with ACS in younger patients, but further studies with longitudinal follow-up protocols are needed to see whether such a causal relationship exists. Although previous studies showed that microbiota-generated metabolites were associated with the development of many cardiovascular diseases (6, 26), to our knowledge this is the first study to investigate the role of the intestinal microbiota in young patients with ACS, showing the important role the intestinal mi-

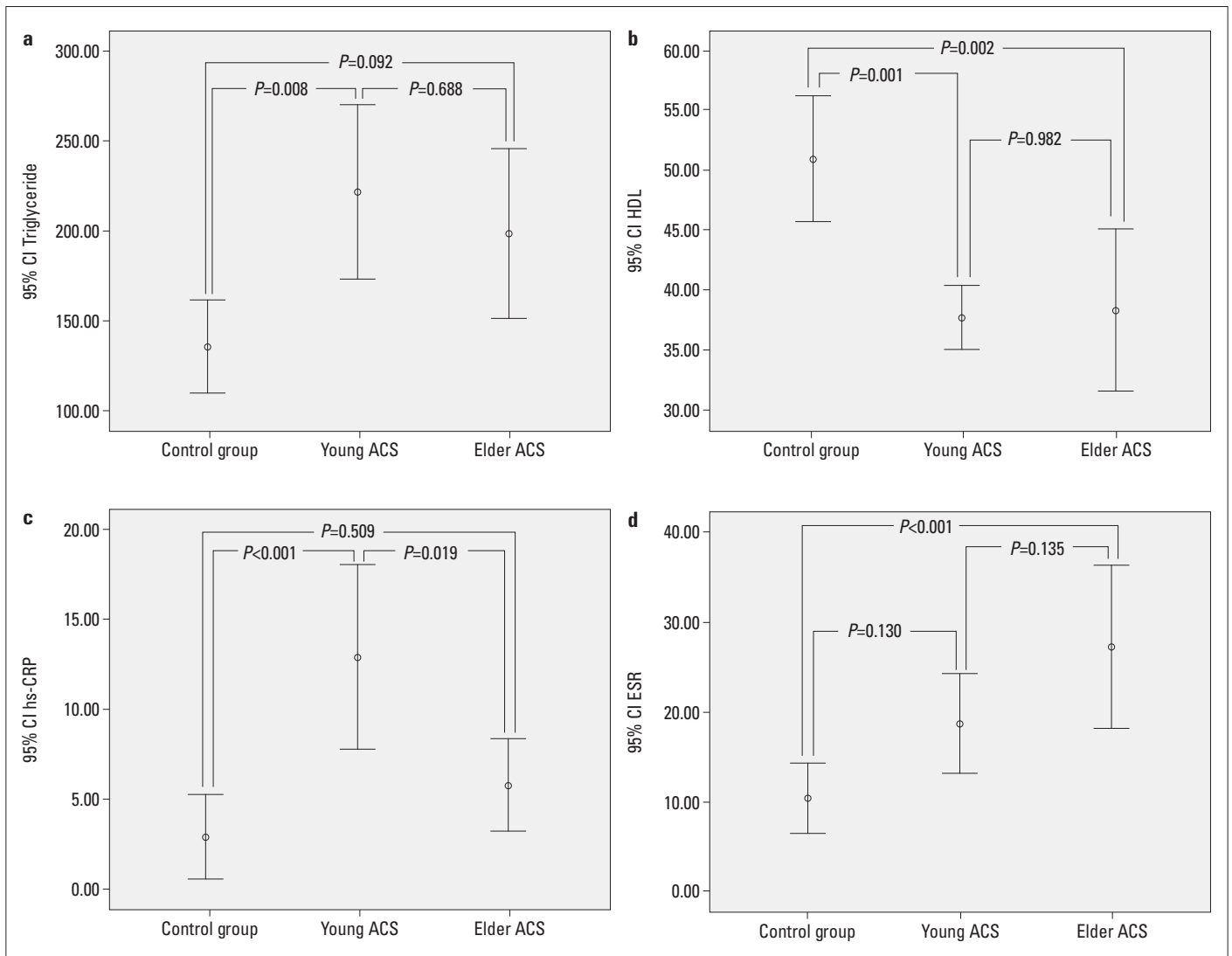


Figure 1. Comparison of the triglyceride (a), HDL-cholesterol (b), hsCRP (c), and ESR (d) values among the groups

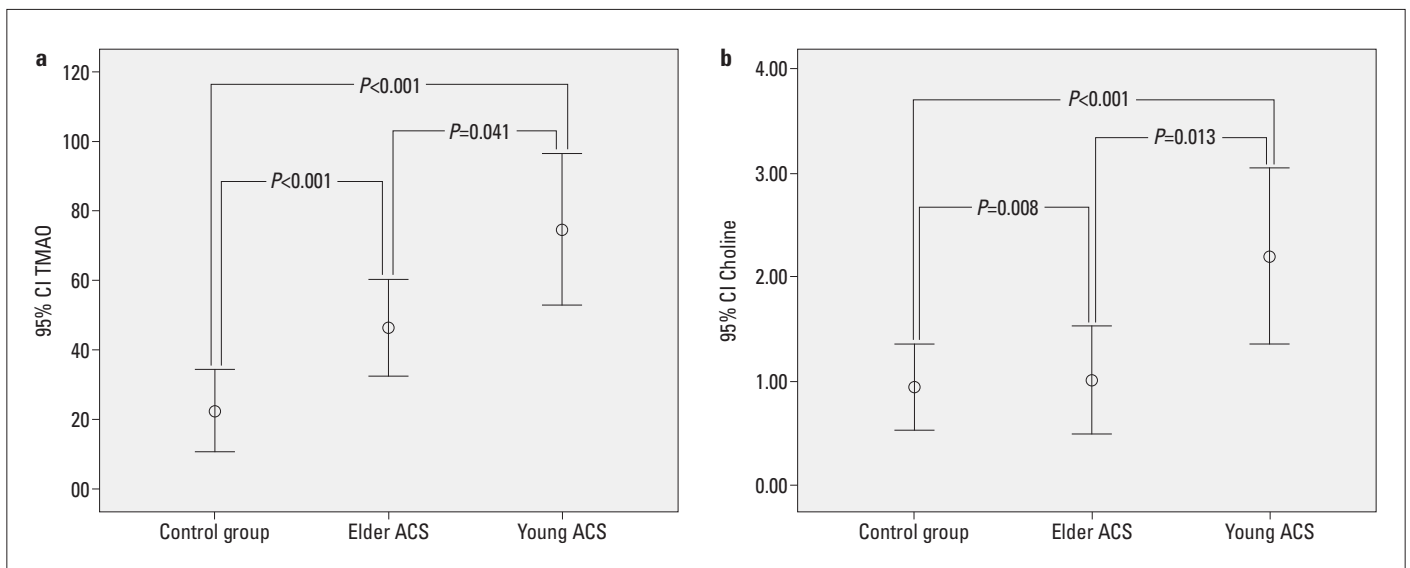


Figure 2. Comparison of the TMAO (a) and choline levels (b) among the groups

crobiota may play in the pathogenesis of young ACS. We believe that future studies that focus on manipulating the microbiota to lessen cardiovascular events may lead to a breakthrough in this area.

Evidence from animal studies supports the concept that the microbiota can mechanistically impact host lipid levels. The possible mechanisms have been explained as follows. First, bile salts in the gut are converted into secondary bile acids by the microbiota. Bile acids entering the blood can activate hepatic or systemic lipid metabolism through certain receptors. The fermentation of nondigestible carbohydrates could be another potential mechanism through which the microbiota could affect lipid metabolism. Short-chain fatty acids, mainly n-butyrate, acetate, and propionate, are produced during the fermentation of these food components and provide rich sources of energy for the host and influence lipid metabolism. Third, TMAOs can directly affect lipid levels by reversing cholesterol transport, cholesterol and sterol metabolism, and/or the degradation of bile acids (27).

CAD is a chronic inflammatory disease. Studies have demonstrated that hsCRP is a good inflammatory biomarker for CV risk classification. This effect of CRP has been reported to be independent from traditional risk factors (28). In this study, we evaluated hsCRP and found significantly higher levels in patients with ACS compared to the control group. In addition, we observed that hsCRP was positively correlated with TMAO. These findings suggest that both TMAO and hsCRP have major clinical importance in patients with young ACS.

In this study, we demonstrated a possible association between microbiota and young ACS. However, it should be taken into consideration that the microbiota varies considerably among individuals. Better understanding of the effect of the microbiota on CAD may provide novel approaches for the prevention of CAD and become a novel potential therapeutic target. Future prospective studies focusing on the microbiota of individuals and even manipulating the microbiota may reduce cardiovascular diseases.

Study limitations

Our study had some limitations, the most significant limitation being its small sample size and single-center nature. Second, we evaluated only baseline TMAO levels and did not perform serial TMAO measurements. Third, we did not investigate the eating habits of the study population. It could be interesting to demonstrate an association between eating habits and TMAO levels. Fourth, although we included a control group in this study, the age of the control group represented only young ACS subjects, not elderly ACS subjects. Adding an elderly control group could be useful. In this study, the control group consisted of young people (age and gender matched). However, the fact that a young control group is used against young ACS may affect the results negatively. Because it cannot be guaranteed that people in the control group will not have a myocardial infarction in the future, therefore, control group consisting of people who have

completed the age limit (above 50 years of age our study) could be more reliable (29). However, we think that performing a two-step regression analysis may partially solve this problem. Finally, we included patients with USAP, NSTEMI, and STEMI in this study. It could be more beneficial to include only patients with acute myocardial infarction to provide a more homogeneous population. Additional prospective studies with larger numbers of participants are required to better explain the relationship between TMAO and young ACS.

Conclusion

The microbiota and its metabolites have recently become a popular research topic. TMAO is the most harmful dead-end metabolite of the microbiota. In this study, we found that young ACS was significantly associated with increased TMAO level.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – M.B.T., Z.T., F.G., İ.K.; Design – M.B.T., Z.T., A.G.; Supervision – M.B.T., F.B., M.E.E., İ.K.; Fundings – M.B.T., Z.T., F.G., F.B., M.E.E., İ.K., A.G., G.G.T.; Materials – M.B.T., F.G., G.G.T.; Data collection and/or processing – M.B.T., Z.T., G.G.T.; Analysis and/or interpretation – M.B.T., Z.T., F.G., F.B., M.E.E., G.G.T.; Literature search – Z.T., F.G., F.B., İ.K.; Writing – M.B.T., Z.T., M.E.E., İ.K.; Critical review – Z.T., F.G., F.B., İ.K., A.G.

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