Systematic Review/Meta-analysis

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Serum trimethylamine N-oxide levels among coronary artery disease and acute coronary syndrome patients: a systematic review and meta-analysis

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Background and Aim: Recent studies have linked trimethylamine N-oxide (TMAO) to cardiovascular diseases; our study aimed to analyze the association between coronary artery disease (CAD), acute coronary syndrome (ACS), and TMAO.

Methods: PubMed, Scopus, Embase, and Web of Science were searched using terms such as 'CAD' and 'TMAO'. Only observational controlled studies were included. RevMan software version 5.4 was used for the analysis.

Results: A significant association was found between the CAD group and increased serum TMAO levels compared with the control group (MD = 1.16, 95% CI = 0.54–1.78, $P = 0.0003$). This association remained significant among acute coronary syndrome patients (MD = 0.98, 95% CI = 0.73–1.23, $P < 0.00001$) and was also detected among young and old CAD patients (MD = 0.35, 95% CI = 0.06–0.64, $P = 0.02$ and MD = 1.36, 95% CI = 0.71–2.01, $P < 0.0001$, respectively). On further analysis of intestinal metabolites, the authors detected an insignificant association between choline, betaine, carnitine, and CAD. According to our sensitivity analysis, TMAO is an acceptable diagnostic marker for CAD (0.721, SE was 0.0816, 95% CI: 0.561–0.881).

Conclusion: TMAO is an acceptable diagnostic marker for CAD, with significantly higher levels among these patients regardless of their age. Other metabolites did not show such an association. The role of serum level TMAO in the early diagnosis of CAD should be further explored.

Keywords: coronary artery disease, gastrointestinal microbiome, microbiota, TMAO

Introduction

In spite of substantial advancements in the realm of preventing and treating coronary artery disease (CAD), cardiovascular incidents continue to remain the primary contributor to both mortality and morbidity in the world[1]. CAD is defined by the gradual narrowing of the epicardial coronary arteries due to atherosclerosis, leading to a reduction in myocardial blood flow[2]. Among the manifestations of CAD, acute coronary syndrome (ACS) is the most dire[3]. ACS refer to a collection of conditions stemming from sudden myocardial ischemia, which spans from unstable angina to myocardial infarction (MI)[4]. Interestingly, certain individuals with significant artery stenosis (equal to or exceeding 90% in coronary arteries) may not display evident clinical manifestations, potentially complicating the diagnosis and prevention of CAD and therefore ACS. Therefore, finding new CAD/ACS biomarkers is crucial given the unmet need for deeper knowledge and effective diagnostic and preventive measures. Recently, several studies have indicated a substantial relationship between the gut microbiota and the pathophysiology and development of CAD[5,6]. Dietary choline,

HIGHLIGHTS

- Coronary artery disease (CAD) patients had higher serum levels of trimethylamine N-oxide (TMAO).
- This association was also observed among acute coronary syndrome patients.
- TMAO is an acceptable diagnostic marker for CAD.
- Choline, betaine, and carnitine did not yield a significant disparity in serum levels among CAD patients compared to the healthy controls.

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betaine, and L-carnitine all contribute to the production of trimethylamine N-oxide (TMAO), a significant intestine microbial metabolite. Trimethylamine (TMA) is produced from it by the microbiota in the gut. Then, it is oxidized by hepatic flavin monooxygenases (FMOs) to form TMAO[7]. Recent research employing genetic modifications of FMO3, the primary enzyme responsible for transforming TMA into TMAO[8], has affirmed the involvement of this meta-microbial pathway in the control of sterol and overall cholesterol metabolism, as well as in the formation of atherosclerotic plaque[9–11]. Elevated levels of TMAO in the bloodstream trigger the influx of cholesterol into macrophages, resulting in the creation of foam cells and, ultimately, the development of atherosclerotic plaques[8]. Hence, heightened TMAO levels have been associated with an elevated likelihood of severe adverse cardiovascular events, as indicated by multiple studies[5,6,12–15]. Apart from the generation of foam cells, alterations in cholesterol and bile acid metabolism, along with the initiation of inflammatory pathways, are believed to underlie the atherogenic effects of TMAO[16]. Several variables, such as age, food, gut microbial flora, medication intake, and liver flavin monooxygenase activity, affect the plasma level of TMAO. It has been discovered that greater levels of TMAO are present in younger individuals with CAD, which may be related to meat consumption[17]. The relationship between TMAO and atherosclerosis has recently been investigated through metabolomics. There have been prior meta-analyses investigating the link between TMAO levels and CAD, with a proposed focus on alterations in the intestinal microbiota as contributors to CAD development. Nevertheless, this study stands out as the initial investigation that takes into consideration patients with ACS and their connection to TMAO levels. Our primary goal was to explore the relationship between plasma TMAO levels and other metabolites involved in TMAO production among individuals with CAD and ACS.

Methods

The protocol of this paper was registered on PROSPERO, and the regulations of the preferred reporting items of systematic reviews and meta-analyses (PRISMA) (Supplemental Digital Content 1, http://links.lww.com/MS9/A285) and AMSTAR (assessing the methodological quality of systematic reviews) guidelines were followed (view the supplemental material for details, Supplemental Digital Content 2, http://links.lww.com/MS9/A286).

Search strategy

Search strategy and literature search

PubMed, Scopus, Embase, and Web of Science were searched from inception till the 6th of May 2022, using key terms such as 'Trimethylamine N-oxide', 'TMAO', 'short-chain fatty acid', 'coronary artery disease', 'angina', and 'acute coronary syndrome' (view the supplementary material for the full search strategy, Supplemental Digital Content 1, http://links.lww.com/MS9/A287).

Inclusion and exclusion criteria

We screened studies by titles and abstracts according to the following:

Inclusion criteria: controlled observational studies reporting data on the serum TMAO levels among adults (≥18 years old) suffering from CAD, including cross-sectional, case–control, and cohort studies.

Exclusion criteria: editorials, commentaries, reviews, systematic reviews, meta-analyses, case reports, case series, animal studies, and studies lacking a control group.

In the case of duplicate studies, the most recent study with the largest study population was included.

Study selection

Two independent reviewers screened the titles and abstracts of the studies according to our criteria. If a consensus was not achieved, a third independent reviewer was assigned to resolve the conflict.

Data extraction and quality assessment

Each study was independently extracted by two reviewers. Data were then compared to confirm accuracy. If a consensus was not achieved, a third independent reviewer was assigned to resolve the conflict.

For the baseline and summary, the following data were extracted: last name of the first author, year of publication, study design, country, sample type, sample size, sex, the prevalence of hypertension, diabetes, and conclusion.

For the outcomes, the following data were extracted: age, serum levels of TMAO, choline, betaine, carnitine, and the area under the curve for the sensitivity analysis.

The risk of bias was assessed utilizing Newcastle–Ottawa Scale (NOS) items[18], with a nine-point score, to evaluate the quality of observational studies. We defined the observational studies with an NOS score of greater than or equal to 7 stars as high quality and an NOS score of less than 7 stars as low-quality.

Definition of controls

Patients with a negative medical history of CAD were allocated to the control group.

Data analysis

The data were analyzed using RevMan software, version 5.4. Sensitivity analysis (leave one out test and subgroup analysis) was used. If there was no detected heterogeneity, the outcomes were analyzed using a fixed-effect model; however, if notable heterogeneity was identified, a random-effect model was employed instead. A mean difference (MD) with a 95% CI was used to present continuous data. Results were considered significant if the *P*-value was less than 0.05.

Results

Literature search

After a complete literature search, 3730 studies resulted, which later became 2458 after the removal of duplicates, eligible for title and abstract screening. Of the 2458, 2186 were irrelevant, and 272 studies were eligible for full-text screening. Finally, eight studies[19–26] were included in the meta-analysis after a full-text screening, as shown in the PRISMA[27] (Fig. 1). A summary of the studies is shown in Table 1. The total number of patients included

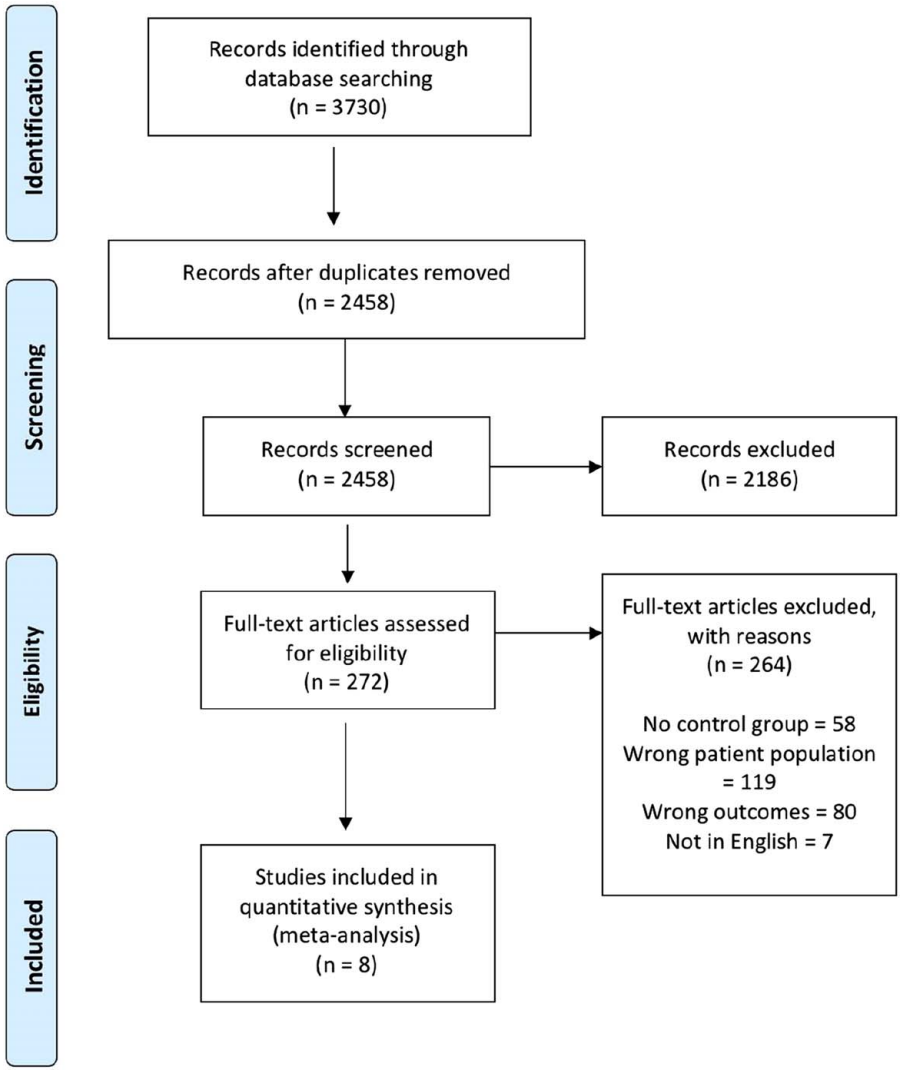

Figure 1. PRISMA flow diagram.

in the study is 2121 patients, 1424 patients in the CAD group and 697 patients in the control group; other baseline data are shown in Table 1. The quality assessment of the included studies is shown in Table 2.

Outcomes

The TMAO level was compared between the CAD group and the control group. Subgroups of less than 65 years and equal to or more than 65 years were conducted according to age. Additionally, we performed a subgroup analysis comparing the TMAO levels among ACS patients and the control group. Different metabolites were analyzed (choline, betaine, and carnitine). Area under curve and standard error were measured by diagnostic test accuracy.

Serum TMAO levels

Our pooled analysis revealed a statistically significant association between the CAD group and increased TMAO level compared with the control group (MD = 1.16, 95% CI = 0.54–1.78, $P = 0.0003$). We detected a significant heterogeneity among studies ($P < 0.00001$, $I^2 = 96\%$) that was not solved by the leave one out test, Fig. 2.

ACS and TMAO

Our pooled analysis revealed a statistically significant association between the ACS group and increased TMAO level compared with the control group (MD = 1.33, 95% CI = 0.62–2.03, $P = 0.0002$). We detected a significant heterogeneity among studies ($P = 0.0006$, $I^2 = 87\%$) that was solved by the leave one out test by removing the study (Dong 2020) ($P = 0.89$, $I^2 = 0\%$), and the analysis showed a statistically significant association between the ACS group and increased TMAO level compared with the control group in the subgroup of TMAO (MD = 0.98, 95% CI = 0.73–1.23, $P < 0.00001$), Fig. 3.

Age subgroup (less than 60 years)

Our pooled analysis revealed a statistically significant association between the CAD group and increased TMAO level

Table 1

Baseline and Characteristics of the included studies.

References	Study design	Country	Sample size	Males, %	Hypertension, %	Diabetes, %	Conclusion
Alhmoud *et al.*[19]	Case–control	USA	38	71	84.2	55.3	Dysbiosis affects patients with ACS, with an increase in intestinal permeability
Bordoni *et al.*[20]	Cross-sectional	Poland	547	65.4	66.5	25.4	No differences between CAD patients and control subjects in plasma TMAO levels
Dong *et al.*[21]	Case–control	China	550	42.4	51.6	N/A	TMAO levels were elevated in both patients with CAD
Dong *et al.*[22]	Case–control	China	298	41.9	N/A	N/A	TMAO is superior to lipoprotein ratios and conventional lipid parameters in predicting occurrence of UAP
Toya *et al.*[23]	Cross-sectional	USA	202	55.9	38.6	12.4	The alteration in circulating levels of OCN-expressing EPCs, which may lead to vascular calcification, may potentially be mediated by gut-microbiome-derived inflammation but not by TMAO
Guo *et al.*[23]	Case–control	China	167	42.5	N/A	N/A	TMAO alone was powerful in risk stratification of CAD and artery stenosis in men; however, in women, no association of TMAO with risk of CAD as well as extent of artery stenosis was observed
Zhong *et al.*[26]	Cross-sectional	China	361	N/A	53.5	29.4	Significant differences in metabolites may be involved in the occurrence and development of atherosclerosis in CAD patients. ROC curve analysis shows that choline and creatinine may yield novel predictive biomarkers that will potentially provide value for clinical diagnosis of CAD
Trøseid [25]	Cross-sectional	Norway	155	82.6	16.8	14.8	The study data show that circulating levels of the microbiota-dependent metabolite TMAO were elevated in patients with chronic HF, and associated with disease severity, ischemic etiology, and reduced survival

ACS, acute coronary syndrome; CAD, coronary artery disease; EPC, endothelial progenitor cells; HF, heart failure; OCN, osteocalcin; ROC, receiver operating characteristic; TMAO, trimethylamine N-oxide; UAP, unstable angina pectoris; USA, United States of America.

compared with the control group in the subgroup of less than 60 years (MD = 0.35, 95% CI = 0.06–0.64, $P = 0.02$). We detected no heterogeneity among studies ($P = 0.73$, $I^2 = 0\%$), Fig. 4.

Age subgroup (equal to or more than 60 years)

Our pooled analysis revealed a statistically significant association between the CAD group and increased TMAO level compared with the control group in the subgroup of equal or more than 60 years (MD = 1.36, 95% CI = 0.71–2.01, $P < 0.0001$). We detected a significant heterogeneity among studies ($P < 0.00001$, $I^2 = 96\%$) that was not solved by the leave one out test, Fig. 4.

Metabolites

Choline

Our pooled analysis revealed no statistically significant difference between the CAD group and the increased choline level compared with the control group (MD = 1.90, 95% CI = – 0.86–4.67, $P = 0.18$). We detected a significant heterogeneity among studies ($P < 0.00001$, $I^2 = 96\%$) that was not solved by the leave one out test, Fig. 5.

Betaine

Our pooled analysis revealed no statistically significant difference between the CAD group and the increased betaine level compared with the control group (MD = 1.61, 95% CI = – 7.48–10.70,

Table 2

Quality assessment of the included studies.

References	Case definition	Representativeness	Selection of controls	Definition of controls	Comparability	Ascertainment of exposure	Same method	Non response rate	Total
Alhmoud *et al.*[19]	1	0	1	1	2	1	1	0	7
Bordoni *et al.*[20]	1	0	1	1	2	1	1	0	7
Dong *et al.*[21]	1	0	0	0	2	1	1	0	5
Dong *et al.*[22]	1	0	0	1	2	1	1	0	6
Toya *et al.*[24]	1	0	0	1	2	1	1	Could not be determined	6
Guo *et al.*[23]	0	0	0	0	2	1	1	Could not be determined	4
Zhong *et al.*[26]	1	0	0	1	0	1	1	Could not be determined	4
Trøseid [25]	1	0	0	1	2	1	1	Could not be determined	6

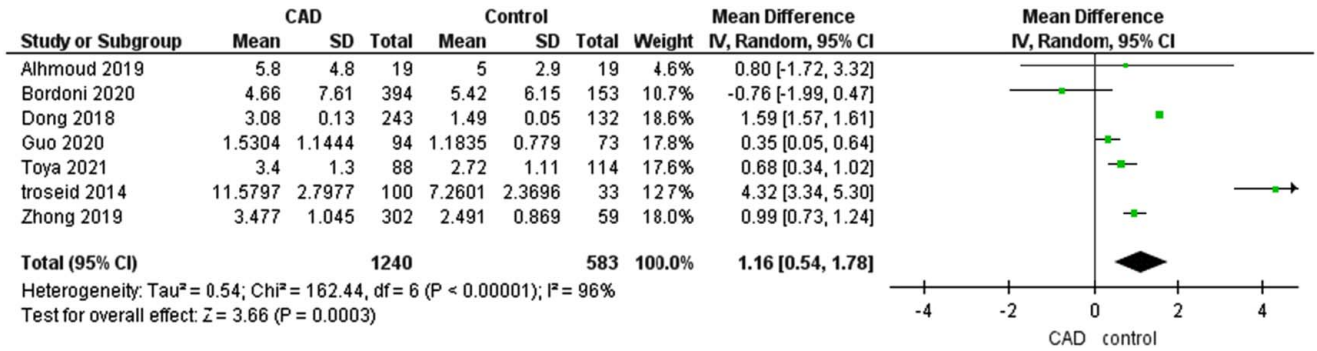

Figure 2. Forest plot of the comparison between the TMAO levels among the CAD group and the control group.

$P = 0.73$). We observed a significant heterogeneity among studies ($P = 0.0001$, $I^2 = 93\%$), Fig. 5.

Carnitine

Our pooled analysis revealed no statistically significant difference between the CAD group and increased carnitine level compared with the control group (MD = 2.31, 95% CI = – 1.73–6.34, $P = 0.26$). We detected no heterogeneity among studies ($P = 0.13$, $I^2 = 57\%$), Fig. 5.

Sensitivity and specificity analysis

Our pooled analysis showed that the overall ROC area for the three included studies was 0.721, SE was 0.0816, 95% CI: 0.561–0.881). We detected significant heterogeneity among studies ($P < 0.0001$, $I^2 = 91.50\%$), Fig. 6.

Discussion

Our meta-analysis compared TMAO levels between people with CAD and the control group, and a significant association between increased TMAO levels and CAD incidence was revealed. Furthermore, ACS incidence was correlated with increased TMAO levels. Further subgroup analysis on the TMAO level was done according to age, which was divided into two subgroups: (less than 60 years old) and (more than or equal to 60 years old) and plasma metabolites, including choline, betaine, and carnitine. The age subgroup analysis showed a significant correlation between the CAD group and an increased level of TMAO compared with the control group in both age subgroups: less than 60 years old and more than or equal to 60 years old. However, the metabolites subgroup analysis revealed no difference between the CAD and control group regarding levels of choline, betaine, and carnitine. In addition, the pooled effect estimate showed that the ROC area for the TMAO level as a biomarker for CAD was = 0.721. This indicates a highly significant value for TMAO as a novel biomarker for CAD.

TMAO is a microbiota-dependent metabolite that is produced mostly by a stepwise mechanism[28]. The synthesis of TMAO from its precursor TMA depends heavily on the human microbiota. The large intestine microbiota can convert carnitine and choline into TMA, which is then broken down by the hepatic enzyme Flavin monooxygenase 3 (FMO3) to produce TMAO[29]. Increased TMAO levels were linked to an elevated risk of several cardiovascular diseases (CVD), including HTN, DM, and atrial fibrillation[31–32], according to previous meta-analyses. However, this is the first meta-analysis to investigate the connection between TMAO levels and CAD, revealing that people with CAD were more likely to have higher levels of circulating TMAO than healthy individuals. Several research studies have demonstrated an association between TMAO plasma levels and cardiovascular risks. According to these studies[14,15,33,34], plasma levels of TMAO were independently linked to a higher risk of significant adverse cardiac events. According to a recent prospective study, major cardiovascular events during a 3-year follow-up were linked to plasma levels of TMAO in patients who underwent selective coronary angiography[16]. TMAO-induced CVDs are explained by various mechanisms, including: (1) Elevated blood TMAO levels trigger macrophage influx of cholesterol, which causes foam cell development and, ultimately, atherosclerotic lesions[28]. (2) Higher TMAO levels have been observed to cause human atherosclerosis through platelet hyperactivity, thrombotic events, EC senescence, vascular aging, and oxidative stress[28]. (3) Endothelial dysfunction induced by vascular

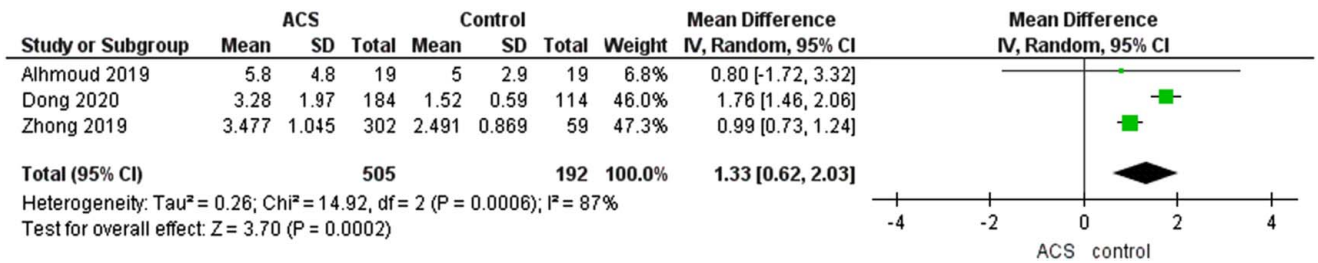

Figure 3. Forest plot of the comparison between the TMAO levels among the acute coronary syndrome group and the control group.

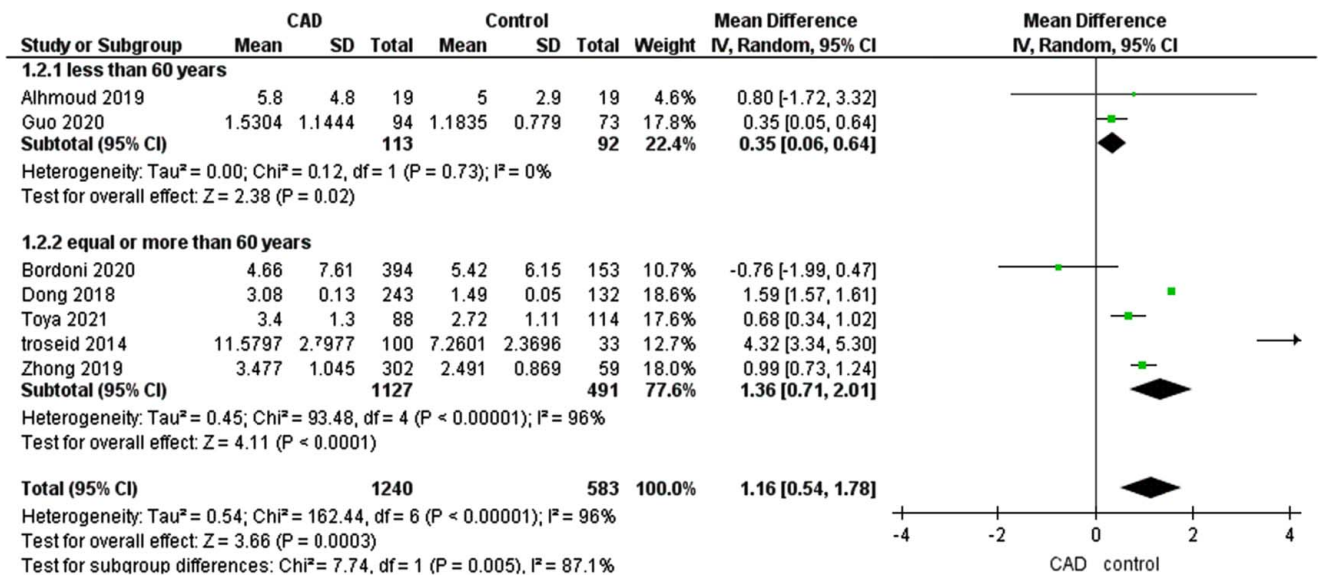

Figure 4. Forest plot of the comparison between TMAO levels according to different age groups.

oxidative stress and inflammation[21]. (4) The elevated fasting serum TMAO level tends to be independent of traditional cardiovascular risk factors such as insulin resistance, visceral obesity, and fatty liver; and is associated with a larger carotid intima-media thickness (cIMT). Additionally, elevated TMAO levels improved endothelial cell-induced white blood cell activation[28]. Additional research into the inhibition of pharmacological and antibiotic interventions revealed that nuclear factor-Kappa B signaling activity was necessary for TMAO to induce inflammatory gene expression in both cell types and leukocyte adherence to endothelial cells[28].

Similar to our results, Guo *et al.*[23] main findings revealed a significant association between fasting plasma TMAO levels in patients with CAD and severe artery lesions. In addition, the high level of TMAO in fasting plasma may act as an independent CAD and severe arterial stenosis predictor. They also discovered comparable results to our analysis, finding no discernible variations in choline, L-carnitine, or betaine concentrations between groups with CAD and healthy controls. In addition, they found that TMAO is more accurate in diagnosing CAD and severe artery stenosis in males compared with women. At the same time, a combination of TMAO, choline, L-carnitine, and betaine also

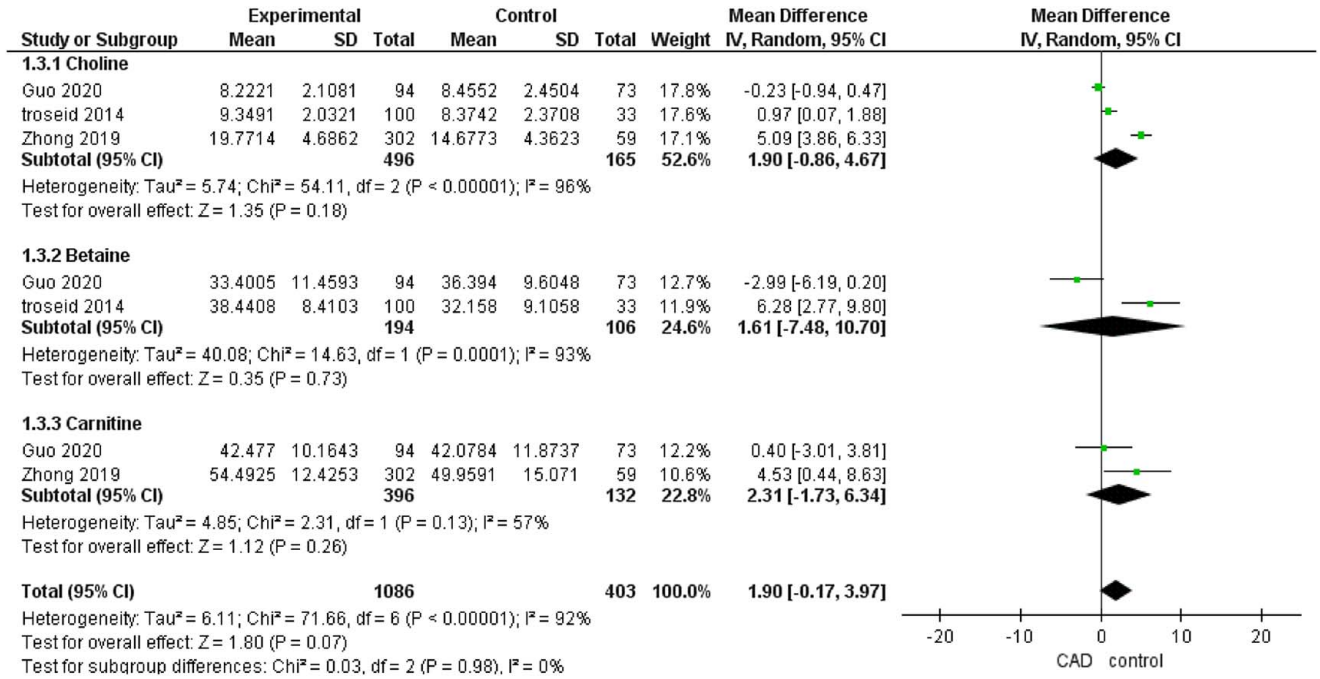

Figure 5. Forest plot of the comparison between different metabolites among the CAD group and the control group.

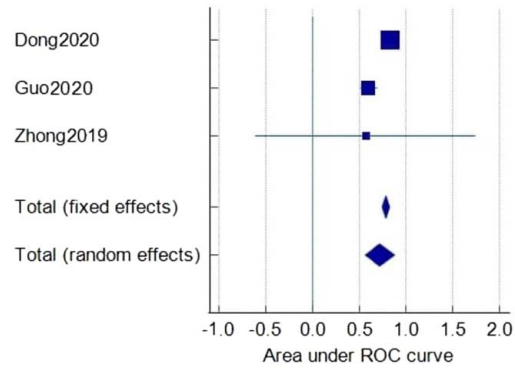

Figure 6. Sensitivity and Specificity analysis.

demonstrated better accuracy in predicting the risk of CAD and severe artery stenosis in both men and women[23]. These variations between men and women may be influenced by dietary habits and microbiome diversity. According to several studies, males tend to consume more red meat and fat and have less fiber-rich diets than women[35–37], which may raise their risk of developing CVD and the deposition of substances containing trimethylamine. Moreover, according to evidence from microbiologists, women may possess a larger ratio of Firmicutes/Bacteroidetes than men[38,39], short-chain fatty acids, which were shown to protect against CVD and may reduce the risk of arteriosclerosis brought on by other gut flora compounds as TMAO, are produced by gut bacteria in the Firmicutes phylum[40]. Guo *et al.* findings could indicate that women's microbiome background, dietary choices, and metabolic or physiologic characteristics may make risk factors like TMAO less effective at predicting the risk of developing CAD. Sex differences should be carefully considered and examined when using TMAO for risk stratification in CAD populations in order to prevent bias in assessments of CAD, particularly in women[13]. Likewise, Zhong *et al.* used targeted metabolomics to examine the plasma concentrations of TMAO in patients with CAD versus healthy controls, finding that the plasma concentrations for TMAO were each significantly higher in patients with CAD than the healthy controls. In contrast to our findings, they discovered that individuals with CAD had considerably greater choline, carnitine, and creatinine plasma concentrations than healthy controls. Choline and creatinine may produce novel predictive biomarkers that could be useful for the clinical diagnosis of CAD, according to ROC curve analysis of the four metabolites[26]. Similarly, Dong *et al.*[21] discovered that in Chinese patients, TMAO was an additional independent predictor beyond the conventional prognostic indicators of coronary heart disease (CHD); in addition, the levels of TMAO were strongly correlated with diabetes in CHD patients. Furthermore, their investigation showed that patients with CHD and CHD-T2DM had higher levels of circulating TMAO than healthy controls. The ability of circulating TMAO to distinguish T2DM patients with CHD from CHD patients and control subjects with an area under the ROC curve is another novel finding in their results. This suggests that circulating TMAO could act as a biomarker for both CHD and the prediction of CHD patients who also have T2DM. Also, they discovered that as compared to CHD patients, CHD-T2DM had significantly higher levels of TMAO[21]. The outcome was in accordance with the earlier investigation[41,42]. In animal and human research, TMAO has also been suggested as a potent candidate molecule to mediate the onset of T2DM[43]. By modifications in the metabolism of cholesterol and bile acids, activation of inflammatory pathways, and stimulation of foam cell production, the elevated levels of TMAO brought on by T2DM may be impacting the development of CHD. The risk of fatal major adverse cardiovascular events and death in CHD-T2DM patients will rise as a result of this event. TMAO was discovered to be a substantial risk factor for Unstable Angina Pectoris (UAP) by Dong *et al.*[22]. Moreover, ROC and logistic regression studies revealed that TMAO was a significant risk factor for predicting UAP in patients beyond lipoprotein ratios and conventional lipid parameters, surpassing both indicators. This finding suggests that measuring TMAO levels can provide important information for determining UAP risk[22]. The key conclusion of the Torseid *et al.*[25] study was that individuals with chronic HF had higher circulating levels of TMAO and its precursors, choline, and betaine. Only TMAO levels were linked to adverse outcomes throughout follow-up, despite the fact that all three indicators were correlated with the severity of the clinical, hemodynamic, and neurohormonal diseases. It may be claimed that the elevated TMAO levels in patients with HF simply reflect coexisting atherosclerotic disease because, in our investigation, the levels of TMAO were highest in HF patients with CAD as the underlying cause. Nevertheless, they found that TMAO levels were considerably higher in individuals with HF of ischemic origin as compared to both individuals with DCM and individuals with stable CAD. The combination of HF and an ischemic etiology appears to be particularly significant, even while an ischemia etiology seems to contribute to the high TMAO levels, and cardiac failure is possibly also implicated[25].

In animal models, suppressing TMAO by methods to block flavin monooxygenase 3—the principal hepatic enzyme responsible for turning microbially produced TMA into TMAO could also prevent atherosclerosis[44,45]. Hence, these findings suggested that TMAO may serve as a viable therapeutic target to treat disease, including individuals with CHD, in addition to appearing to be a risk sign.

In contrast to our findings, Bordoni *et al.* discovered no differences in plasma TMAO levels between CAD patients and healthy controls. Surprisingly, they found that TMA levels were lower in CAD patients compared to controls[20]. Similarly, Alhmoud *et al.*[19] discovered no significant variation in TMAO levels between the ACS and healthy control groups.

On examining the relationship between age and TMAO levels, several studies provide compelling insights. Chen X *et al.*, observed a significant rise in plasma TMAO levels in individuals aged 65 and above, which partially aligns with our findings indicating higher mean plasma TMAO levels in the subgroup of individuals aged 60 or older[46]. Similarly, Li Dang, and their colleagues reported analogous results, revealing a substantial increase in plasma TMAO levels among elderly participants (aged ≥ 65) compared to both younger and middle-aged groups. However, no noticeable variations in circulating TMAO levels were observed between the young and middle-aged groups[47].

Furthermore, our exploration of the association between TMAO levels in CAD and age reveals mixed results. Guo *et al.* found no statistically significant link between TMAO levels in CAD and age[23], while Trozeid *et al.* obtained results consistent with ours, showing a statistically significant association between TMAO levels in CAD patients and age[25]. Multiple controlled

prospective observational studies are warranted to examine the association between different age groups and TMAO levels among CAD/ACS patients.

Future implications

Our analysis findings have demonstrated the utility of TMAO as a potential diagnostic and prognostic biomarker for CAD/ACS, as well as a preventive indicator for CAD-related complications, such as ACS. TMAO plays a multifaceted role in the promotion of atherosclerosis (AS), thereby contributing to the development of CAD and its associated complications. These roles encompass various aspects: immunity role, as extensive research on the mechanisms of AS has unveiled that immune activation has a net proatherogenic effect. Consequently, there is a valid argument for considering AS, at least to some extent, as an autoimmune disease[48]. Notably, TMAO levels exhibited significant correlations with the proportion of proinflammatory intermediate monocytes in patients with ischemic stroke[49]. In HIV patients with carotid AS, TMAO displayed positive associations with two biomarkers indicative of monocyte activation and inflammation[50]. Furthermore, TMAO activated macrophage expression, leading to enhanced uptake of ox-LDL and the formation of foam cells[5,51]. An inflammatory role, as elevated plasma TMAO levels were observed in obese mice, coinciding with increased expression levels of proinflammatory cytokines, including TNF-α and IL-1β, while exhibiting decreased expression of the anti-inflammatory cytokine IL-10[52]. Cholesterol metabolism, as the TMA/FMO3/TMAO pathway, driven by the gut microbiota, plays a pivotal role in regulating lipid metabolism[8,11]. Atherosclerosis and thrombosis, as TMAO contributes to platelet hyperreactivity, thereby elevating the potential for thrombosis[12,53]. These factors are associated with the prospective risk of coronary events, mortality, and the extent of terminal organ damage, such as myocardial injury[54–57].

TMAO as a potential diagnostic biomarker: evidence suggests that urinary TMAO levels are correlated with the risk of CHD and may expedite its development[58]. Moreover, in patients experiencing ST-segment elevation myocardial infarction (STEMI), elevated plasma TMAO levels have demonstrated predictive value for both a high SYNTAX score and the presence of multivessel disease. These measures are employed to assess the extent of coronary atherosclerotic burden. Consequently, TMAO has been linked to an increased burden of coronary atherosclerosis in STEMI patients[59]. Furthermore, a 3-year follow-up study involving 4007 patients undergoing elective coronary angiography has uncovered a significant association between elevated plasma TMAO levels and an elevated risk of stroke, MI, or mortality[6]. In addition to our findings, TMAO levels can serve as an initial diagnostic biomarker for CAD, in conjunction with considering the clinical context and comorbidities, which can then be complemented by additional laboratory and imaging studies. Further research is warranted to assess the sensitivity and specificity of TMAO level analysis and compare it to currently established diagnostic tests.

TMAO as a potential prognostic biomarker: among patients experiencing chest pain and ACS, elevated plasma TMAO levels have demonstrated the ability to forecast both short-term (30-day/6-month) and long-term (1–7-year) cardiovascular event risks[60,61]. In individuals with stable CAD[62], heart failure[15], and peripheral artery disease (PAD)[63], increased TMAO levels have been identified as predictors of 5-year mortality, with corresponding elevated mortality risks of 4-fold, 3.4-fold, and 2.7-fold, respectively. Our study findings affirm that higher TMAO levels exhibit statistically significant associations with CAD and ACS. Consequently, TMAO can serve as a prognostic biomarker for mortality or the risk of cardiovascular events, complementing existing mortality assessment tools for CAD (such as the polygenic risk score for CAD[64]) or ACS (such as the TIMI score[65]). Further investigations are warranted to compare current prognostic scoring systems with TMAO level analysis, enabling a comprehensive assessment of the potential impact of this biomarker on the morbidity and mortality of CAD/ACS patients.

Moreover, TMAO levels can serve as a biomarker to initiate stronger preventive strategies against CAD complications, such as ACS, while also reducing the risk of mortality in CAD patients. Given the statistically significant association we have established between TMAO levels and CAD/ACS, proactive steps can be taken, particularly in terms of dietary modifications.

The primary dietary precursors of gut microbe-dependent TMAO, including choline, L-carnitine, betaine, and other choline-containing compounds, are abundant in the human diet[66,67]. It is advisable to counsel and guide patients toward adopting a Mediterranean diet (MD), which has demonstrated positive effects in lowering TMAO levels, as well as reducing cardiovascular risk and overall mortality[68–70]. Conversely, it is important to discourage the consumption of high-fat or western-style diets, which have been shown to elevate plasma TMAO levels in both human and animal studies[52,71].

Furthermore, it is prudent to advise against excessive consumption of L-carnitine-rich foods, such as red meat, as this dietary pattern has been linked to accelerated atherosclerosis due to alterations in microbial composition and increased production of TMA and TMAO[66,72]. For instance, long-term data from a prospective study involving 84 136 women over 26 years revealed that high intakes of red meat significantly increased the risk of CHD R34.

The role of gut microbiota in TMAO generation is pivotal[5,66], making it essential to address microbial composition. Patients with large-artery atherosclerotic stroke and transient ischemic attacks often exhibit noticeable intestinal dysbacteriosis[73]. In germ-free mice, insufficient colonization of TMA-producing bacteria has led to a significant accumulation of plasma TMAO[74]. Utilizing probiotic functional products represents a potentially safer and more effective means of reshaping the microbiota composition. In certain animal experiments, the administration of probiotic strains like Lactobacillus plantarum[75] and Enterobacter aerogenes[76] has substantially reduced choline-induced cecal TMA and serum TMAO levels by modulating gut microbiota; however, it is worth noting that probiotics proven to effectively lower TMAO levels in human studies remain relatively limited[77,78].

Additionally, advocating lifestyle interventions can exert an impact on plasma and urine TMAO levels. For instance, among a group of 16 obese adults, a 12-week regimen of a hypocaloric diet coupled with exercise yielded a notable reduction in the percentage change in TMAO compared to a eucaloric diet and exercise regimen[79].

However, it is important to acknowledge that inhibiting TMAO may have potential adverse consequences. For instance, the suppression of FMO3 expression can lead to the substantial accumulation of TMA, resulting in a condition known as trimethylaminuria, colloquially referred to as 'fish odor syndrome,' which can significantly impact a patient's quality of life[80,81]. Furthermore, considering that FMO3 plays a systemic role in

catecholamine metabolism, inhibiting its function may not be without consequences, given that the genetic absence of FMO3 activity has been linked to hypertension[82].

In summary, we propose that TMAO levels could potentially offer a novel approach for diagnosing and prognosticating CAD/ACS, either alone or in conjunction with existing risk assessment tools. Additionally, TMAO may serve as a preventive biomarker for complications associated with CAD, such as ACS. Nevertheless, further research is essential to determine the specific plasma TMAO levels indicative of elevated risk and to establish the correlation between metabolite concentrations and an increased risk of cardiovascular events and ACS.

Strengths and limitations

In the majority of the studies we included in our analysis, the overall quality was satisfactory. Furthermore, it is worth noting that our meta-analysis is, to the best of our knowledge, the first to conduct a subgroup analysis based on age, which is a crucial factor influencing variations in TMAO levels, as previously mentioned. It is important to acknowledge that most of the studies we incorporated were observational in nature. Therefore, to reinforce our findings and to further investigate the relationship between TMAO and CAD, there is a need for prospective multicentric studies.

An additional limitation lies in our inability to run sensitivity analyses on potentially confounding variables such as diet, medication usage, comorbidities, or lifestyle factors. This limitation stems from the lack of information available in the majority of the studies we analyzed. Also, some of the included studies in the analysis received low-quality assessment scores. More studies are warranted to address these limitations.

Conclusion

Our study showed a significant association between the CAD/ACS group and increased microbiota-dependent metabolite TMAO levels compared with the control group. However, the CAD group and the control group did not significantly differ, according to our pooled study regarding levels of choline, betaine, and carnitine. These results imply the prospective clinical applicability of TMAO in the context of CAD and ACS. Consequently, we propose that TMAO could serve as a valuable diagnostic and prognostic indicator for CAD and ACS, holding substantial promise as a future biomarker of significance. Additionally, to validate our conclusions, it is essential to carry out meticulously planned experimental investigations that delve into the influence of TMAO and other microbiota-related elements on the mechanisms underlying CAD's and ACS' development.

Ethical approval

Ethics approval was not required for this review and meta-analysis.

Consent

Informed consent was not required for this review and meta-analysis.

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Author contribution

Y.E.D.: conceptualization; D.G., S.V., and Z.K.: screening; Z.H., A.H., and Z.K.: data extraction; N.E.T.: statistical analysis; S.S.R., Y.E.D., A.R., A.F., and T.M.: writing; Y.H. and H.A.: supervision. All authors reviewed the final manuscript and approved it.

Conflicts of interest disclosure

All authors declare no conflict of interest.

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Guarantor

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Data availability statement

Data is available upon reasonable request to the corresponding author.

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