The Relationship of Serum Trimethylamine N-Oxide Levels with Carotid Intima-Media Thickness and Disease Activity in Psoriasis Patients

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ABSTRACT

Introduction: Psoriasis is an inflammatory disease that can cause cardiovascular comorbidities. Some recent studies have indicated that impaired gut microbiota and metabolites may be associated with inflammatory diseases.

Objectives: In this study, the relationship between serum trimethylamine n-oxide (TMAO, a gut bacterial metabolite) level and carotid intima-media thickness (CIMT) and disease severity in psoriasis patients was investigated.

Methods: Age- and gender-matched 73 patients and 72 healthy controls were included in the study. In both groups serum trimethylamine n-oxide(TMAO), oxidized low- density lipoprotein (ox-LDL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, total cholesterol, high-sensitivity C-reactive protein (hs-CRP), creatinine, aspartate aminotransferase (AST) and alanine aminotransferase(ALT) levels were recorded and the carotid intima-media thickness (CIMT) was measured by B-mode ultrasonography by a cardiologist.

Results: TMAO, hs-CRP, oxidized-LDL, triglyceride and CIMT levels were statistically higher in the patient group. HDL levels were statistically higher in the control group. There was no significant

difference between the two groups in terms of total cholesterol and LDL-C levels. In partial correlation analyzes in the patient group, positive correlations were observed between TMAO and CIMT, LDL-C and total cholesterol levels. Linear regression analysis showed that TMAO levels positively predicted CIMT levels.

Conclusions: This study confirmed that psoriasis is a risk factor for the development of cardiovascular disease and that elevated serum TMAO levels in these patients indicate the presence of intestinal dysbiosis. Furthermore, TMAO levels were found to be a predictor of the risk of developing cardiovascular disease in psoriasis patients.

Introduction

Psoriasis is a chronic inflammatory disease managed by the immune system. Systemic inflammation that psoriasis causes can affect many organs and systems [1]. Effector T lymphocytes such as TH1 and TH17 are involved in the pathogenesis of psoriasis and atherosclerosis. Although the mechanisms underlying the relationship between these two diseases are still poorly understood, the inflammatory cytokine profile that plays a fundamental role in both diseases seems to provide a common pathogenic basis [2].

TMAO (oxidized product of trimethylamine) is an intestinal microbial metabolite. Most of the trimethylamine produced from the metabolism of choline and L-carnitine by intestinal bacteria is absorbed into the bloodstream and oxidized to TMAO by the flavin-containing monooxygenase-3 (FMO-3) enzyme in the liver [3]. Increased serum TMAO concentration is associated with intestinal dysbiosis [4]. Remarkably, a dysbiotic microbiome similar to the dysbiosis detected in individuals with elevated serum TMAO levels has also been demonstrated in psoriasis patients [5,6].

Cross-sectional and prospective studies show a positive association between elevated plasma TMAO levels and increased risk for major cardiovascular events. These studies demonstrated a positive correlation between circulating TMAO levels and carotid intima-media thickness after controlling for strong predictors of cardiovascular disease including age, sex and visceral fat mass [7,8].

Due to similar laminar flow properties, imaging of the carotid arterial system can provide information about coronary atherosclerosis. Carotid intima-media thickness (CIMT) measured by B-mode ultrasonography can reliably demonstrate changes in the arterial wall [9,10].

Objectives

In this study, we aimed to compare serum TMAO levels in psoriasis patients and healthy participants and to determine the relationship between TMAO levels and CIMT and other atherosclerotic risk factors in atherosclerotic disease risk prediction.

Methods

Patients and Controls

Seventy-three patients diagnosed with plaque psoriasis (clinically and histopathologically) and 72 healthy participants as the control group were included in the study. An informed consent form was obtained from all participants and they were informed about the study. The study was conducted between 01.06.2020 and 01.06.2021.

The exclusion criteria were determined as follows:

- Special dietary practices, continuous use of prebiotics and probiotics
- Consumption of >2 eggs per day, meat and fish >3 times per week
- Use of any medication that may adversely affect the microbiota in the last 1 month
- Those diagnosed with diseases that may affect the microbiome such as inflammatory bowel diseases, irritable bowel disease
- Those diagnosed with diabetes mellitus, hypertension, hypercholesterolemia and/or taking antidiabetic drugs, antihypertensive drugs, antihyperlipidemic drugs
- Thyroid disease, cardiovascular and cerebrovascular diseases, peripheral vascular disease and systemic vasculitis, chronic kidney/liver disease, systemic infectious disease, collagen tissue disease and/or antiaggregant/anticoagulant drug users
- Use of methotrexate, cyclosporine, biologic agents, systemic steroid and hormonal therapy in the last 3 months
- Malignancy, pregnancy, breastfeeding
- Patients under 18 years of age
- Obesity, smokers, drinkers

Demographic information of all participants was recorded and systolic/diastolic blood pressure was measured from the brachial artery after 5 minutes of rest. Body-mass index (BMI) of all participants and psoriasis area severity index (PASI) of patients were calculated and recorded. TMAO, oxidized-LDL, hs-CRP, cholesterol panel, ALT-AST, creatinine, ALT-AST, creatinine levels were determined in serum samples obtained after 12 hours of fasting. Right and left carotid intima-media thicknesses were measured and recorded in all participants.

Measurement of Biochemical Markers

Blood samples were collected from the antecubital regions of the participants into gel propylene tubes using a vacutainer. Blood samples were allowed to clot for 20 minutes, centrifuged at 3000 rpm for 10 minutes and stored at -80°C until the study day. Frozen serum samples were kept at room temperature on the study day and the samples were thawed.

MyBioSource brand Oxidized LDL (catalog No: MBS265658) commercial kit for oxidized-LDL analysis and MyBioSource brand TMAO (catalog No: MBS7254766) commercial kits for TMAO analysis were used. Analyzes were performed using Rayto RT-2600Microplate Washer and BMG LABTECH Enzyme-Linked ImmunoSorbent Assay (ELISA) reader. The quantitation limits for the oxidized LDL kit are 31.2-2000 pg/mL, and the quantitation limits for TMAO are 0-100 ng/mL. Samples were diluted 1/10 before the study.

ALT, AST, creatinine, total cholesterol, triglyceride, HDL-cholesterol, total cholesterol, triglyceride, HDL-cholesterol levels were analyzed by spectrophotometric method on Beckman Coulter AU5800 Series using Beckman Coulter commercial kits.

Hs-CRP levels were analyzed by immunoturbidimetric method using Beckman Coulter brand commercial kits in Beckman Coulter AU5800 Series device.

Measurement of Carotid Intima-Media Thickness

Carotid artery ultrasound to measure carotid intima-media thickness was performed by a cardiologist using a commercially available ultrasound system (Vivid E9, GE Vingmed) and a linear transducer probe (11L-D, 5-12 MHz).

Participants were examined in the supine position after resting for at least 5 minutes.

Imaging of both arteria carotids communis was performed with the participants' head in a slightly extended and slightly retracted position with the carotid bifurcation as the reference point.

Two-dimensional, real-time, grayscale images in the longitudinal plane were acquired at frame rates of 20-50 fps with focus and gain settings adjusted to maximize visibility of the near and far wall of the artery.

Statistical Method

All data were analyzed in computer environment using SPSS 22.0 package program. Categorical data were evaluated with the Chi-Square Exact test. The Kolmogorow-Smirnow test was performed to determine whether the continuous data showed a normal distribution. Continuous data that did not show normal distribution were tested for their conformity to the normal distribution by data transformation. In the comparison of continuous data of two independent groups, Student-t test was used when parametric test conditions were met, Mann Whitney U test was used when parametric test conditions were not met. In order to determine the relationship between continuous variables, Pearson correlation test was used when normality conditions were met and Spearman correlation test was used when normality conditions were not met. In order to determine the true relationship between the data, the partial correlation test was used by controlling the related variables. Simple and multiple linear regression analysis was applied to determine the risk of cardiovascular disease. Regression analysis data were reported as R square, adjusted R square, standardized coefficiency β coefficients. In the analysis of all hypothesis tests, the level of significance (P value) was accepted as 0.05.

Results

The patient and control groups were similar in terms of gender.

There was no significant difference between the patient and control groups in terms of mean age, BMI, systolic/diastolic blood pressure($P > 0.05$).

TMAO (323.34 ± 240.36 ng/ml versus 220.96 ± 85.36 ng/ml), hs-CRP (2.318 ± 2.00 mg/L versus 1.306 ± 0.74 mg/L), oxidized-LDL (82.50 ± 42.03 pg/ml versus 61.34 ± 31.34 pg/ml) and triglyceride (150.87 ± 87.85 mg/dl versus 119.83 ± 70.02 mg/dl) levels were higher in the patient group compared to the control group (Figure 1).

There was no significant difference in total cholesterol and LDL-cholesterol levels in the patient and control groups($P > 0.05$).

HDL-cholesterol levels were higher in the control group compared to the patient group (47.75 ± 10.12 mg/dl versus 39.09 ± 9.38 mg/dl).

Left-anterior (0.598 ± 0.129 nm versus 0.534 ± 0.068 nm), left-posterior (0.602 ± 0.172 nm versus 0.528 ± 0.093 nm) and right-anterior (0.623 ± 0.177 nm versus 0.537 ± 0.086 nm), right-posterior (0.605 ± 0.164 nm versus 0.520 ± 0.075 nm)
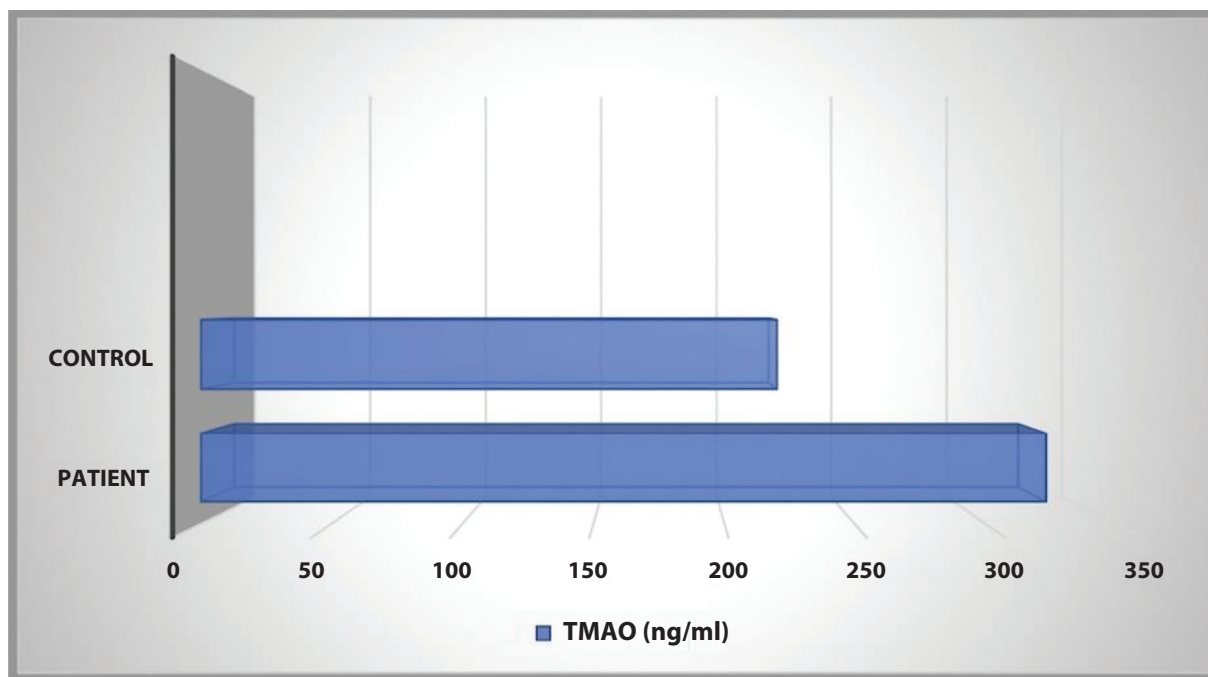
Figure 1. Trimethylamine n-oxide levels in patient and control groups.

carotid intima-media wall thicknesses were significantly higher in the patient group compared to the control group (Figures 2 and 3).

The clinical and laboratory data of the patient and control groups are given in Table 1.

In the partial correlation analysis performed after controlling for age, disease duration and BMI parameters, positive correlations were observed at various levels between TMAO levels and total cholesterol, LDL cholesterol, left anterior and right posterior CIMT (Table 2).

When parameters that may affect CIMT (age, BMI, disease duration, PASI score, blood cholesterol, hs-CRP and oxidized-LDL) were controlled, positive correlations were found between TMAO levels and left anterior and right posterior CIMT at various levels (Table 3).

Simple linear regression analysis was performed to predict the left CIMT variable by using TMAO levels. A significant regression model was found in which 10% (R square adjusted = .10) of the variance in the left CIMT was explained by the independent variable (F [1, 71]:8.084, P = 0.006). Accordingly, TMAO predicted left CIMT positively and significantly, ß= .32, t(71) = 2.843, P = :0.006 (Table.4).

Multivariate linear regression analysis was performed to predict the left CIMT variable by using TMAO, PASI, disease duration, age, ox-LDL, total cholesterole, triglyceride, BMI variables. A significant regression model was found in which 41% (R square adjusted= .41) of the variance in the left CIMT was explained by the independent variables (F [9, 63]:6,644, P < 0.001). Accordingly, TMAO predicted left CIMT positively and significantly (ß= .371, t(63)=3.801,

P < 0.001). In addition, age predicted left CIMT positively and significantly (ß= .618, t(63)=5.428, P < 0.001). Oxidized-LDL, hs-crp, triglyceride, total cholesterol, PASI, disease duration and BMI did not significantly predict left CIMT in this model (P > 0.05). The regression model confirmed that TMAO was a positive predictor for left anterior CIMT and may be a predictor for cardiovascular disease secondary to these outcomes (Table 5).

Conclusions

Psoriasis is not only a skin and joint disease, but also a systemic inflammatory disease that can be associated with various comorbidities. It is associated with an increased risk of developing serious vascular events, particularly myocardial infarction and stroke. Psoriasis and atherosclerotic cardiovascular disease share common genetic and pathophysiological pathways, including genetic factors, inflammatory pathways, secretion of adipokines, insulin resistance, lipoprotein composition and function, angiogenesis, oxidative stress and hypercoagulation [11].

The development of atherosclerosis is a major pathological process that can lead to myocardial infarction and stroke. In the literature, there are studies showing that arterial wall thickness is increased in patients with psoriasis compared to healthy controls and this increase is correlated with disease severity. Positron emission tomography/computed tomography studies have found that aortic wall inflammation is higher in patients with psoriasis than in the healthy population and that there is a positive correlation between disease
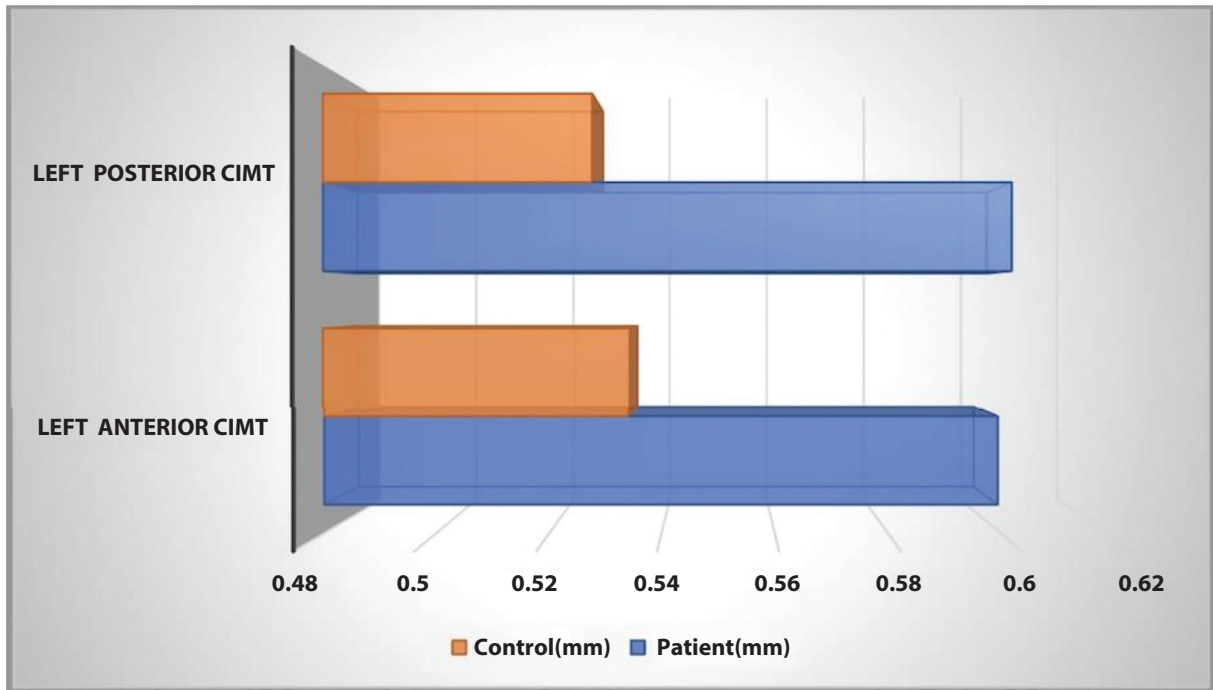
Figure 2. Left carotid intima-media thickness levels in patient and control groups.
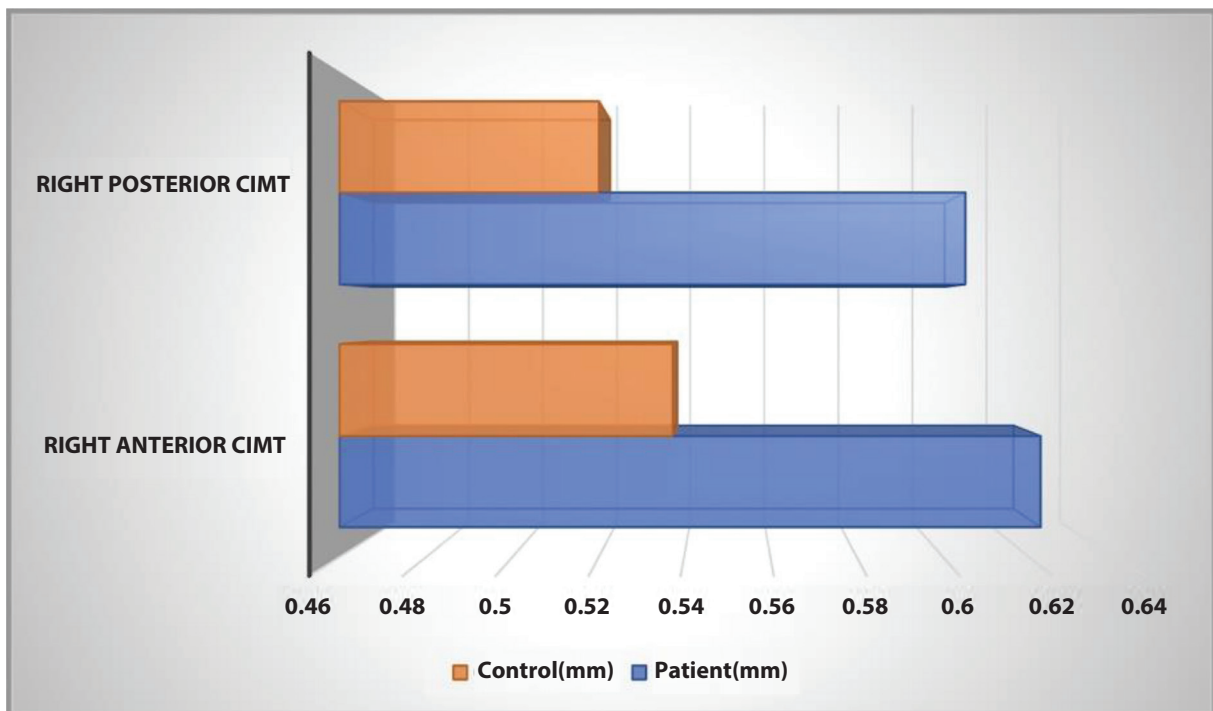
Figure 3. Right carotid intima-media thickness levels in patient and control groups.

severity and inflammation severity. Furthermore, aortic inflammation decreases with healing of skin lesions [12,13].

The finding of higher CIMT levels in psoriasis patients compared to healthy controls in carotid artery ultrasonography studies clearly demonstrates an increased risk of cardiovascular disease in psoriasis patients [14-16].

Our study showed that CIMT levels were significantly higher in psoriasis patients compared to the control group. There were also positive correlations between CIMT values and patient age, disease duration, TMAO, hs-CRP, triglyceride, total cholesterol, body mass index and blood pressures. In the light of these data, CIMT measurement can be used

Table 1. Data for patients and healthy controls.

	Patient (N = 73) Mean ± SD	Control (N = 72) Mean ± SD	Pa
Disease type	Type-1(%86.30) Type-2(%13.70)		
Age (year)	41.57 ± 12.98	41.22 ± 9.25	0.851
BMI (kg/m^2)	26.14 ± 4.20	25.63 ± 3.35	0.420
Systolic blood pressure (mmHg)	121.34 ± 10.05	122,06 ± 10.50	0.676
Diastolic blood pressure (mmHg)	79.74 ± 7.27	81.26 ± 7.62	0.187
TMAO (ng/ml)	323.34 ± 240.36	220.96 ± 85.36	0.028
HsCRP (mg/L)	2.318 ± 2.00	1.306 ± 0.74	0.013
Oxidized-LDL (pg/ml)	82.50 ± 42.03	61.34 ± 31.34	0.010
Total cholesterol (mg/dl)	189.77 ± 41.12	194.29 ± 34.71	0.475
HDL cholesterol (mg/dl)	39.09 ± 9.38	47.75 ± 10.12	0.001
LDL cholesterol (mg/dl)	120.74 ± 36.71	121.99 ± 29.68	0.323
Triglyceride (mg/dl)	150.87 ± 87.85	119.83 ± 70.02	0.025
AST (IU/L)	20.50 ± 5.53	20.93 ± 4.27	0.291
ALT (IU/L)	17.13 ± 8.18	17.82 ± 7.95	0.523
Creatinine (mg/dL)	0.75 ± 0.14	0.75 ± 0.20	0.948
Left anterior CIMT (mm)	0.598 ± 0.129	0.534 ± 0.068	0.010
Left posterior CIMT (mm)	0.602 ± 0.172	0.528 ± 0.093	0.030
Right anterior CIMT (mm)	0.623 ± 0.177	0.537 ± 0.086	<0.001
Right posterior CIMT (mm)	0.605 ± 0.164	0.520 ± 0.075	0.020

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CIMT = carotid intima-media thickness; HsCRP = high sensitivity C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; SD = standard deviation; TMAO = trimethylamine n-oxide.

aThe level of significance (P value) was accepted as 0.05.

to detect the risk of early atherosclerotic disease in patients with psoriasis, which is consistent with previous studies.

Microbiota related immunomodulation has shown that the gut microbiome plays a role in influencing distant organs, mucosal and hematopoietic immune function. Disruption in the composition and function of the intestinal microbiota is associated with a variety of chronic diseases, from gastrointestinal inflammatory and metabolic diseases to neurological, cardiovascular and respiratory system diseases. The changes observed in the composition of the gut microbiome in studies with psoriasis patients have prompted researchers to investigate the molecular mechanisms associated with the microbiome and its possible impact on the disease [17-19].

In recent years, the TMAO molecule formed by the oxidation in the liver of TMA produced from choline and L-carnitine by the gut microbiome is thought to be associated with increased cardiovascular risk and atherosclerosis, but there are also conflicting results.

The dysbiotic gut microbiome observed in psoriasis patients is similar to the composition of the dysbiotic microbiome, which is involved in TMAO production [5,6,17]. TMAO

plays an important role in oxidized-LDL accumulation and foam cell formation within macrophages by increasing the expression of CD36 and macrophage scavenger receptors (SR-A1). It enhances macrophage chemotaxis, expression of inflammatory cytokines including TNF-alpha and IL-6, and may exacerbate inflammation via MAPK and NF-κB. It also decreases the expression of the anti-inflammatory cytokine IL-10 [20-23].

Studies investigating serum TMAO levels and their relationship with comorbidities in patients with psoriasis are still very limited. Sikora et al. found that TMAO levels were significantly higher in psoriasis patients than in controls and this elevation was in parallel with the increased cardiovascular risk [24].

In this study, we found that serum TMAO levels were significantly higher in psoriasis patients compared to the control group. In the patient group, there were positive correlations between serum TMAO concentrations and CIMT values when other cardiovascular risk markers were controlled. The results support the presence of gut dysbiosis in psoriasis patients, and these data support the idea that

Table 2. Correlations when controlling for age, BMI, and disease duration in the patient group.

		TMAO	Oxidized-LDL	Hs-CRP	Triglyceride	Total-C	HDL-C	LDL-C	PASI	Left ant CIMT	Left post CIMT	Right ant CIMT	Right post CIMT
TMAO	P	1.000											
	r	0.000											
Oxidized-LDL	P	0.667	1.000										
	r	0.052	0.000										
HsCRP	P	0.510	0.847	1.000									
	r	-0.080	0.023	0.000									
Triglyceride	P	0.821	0.022	0.265	1.000								
	r	-0.027	0.274	0.135	0.000								
Total-C	P	0.006	0.465	0.748	0.003	1.000							
	r	0.325	0.089	-0.039	0.345	0.000							
HDL-C	P	0.655	0.573	0.014	0.001	0.903	1.000						
	r	0.054	-0.069	-0.293	-0.387	0.015	0.000						
LDL-C	P	0.003	0.960	0.804	0.837	<0.001	0.559	1.000					
	r	0.349	-0.006	-0.030	0.025	0.918	-0.071	0.000					
PASI	P	0.580	0.288	0.613	0.380	0.114	0.511	0.129	1.000				
	r	-0.067	-0.129	0.062	-0.106	-0.191	0.080	-0.183	0.000				
Left ant CIMT	P	<0.001	0.340	0.547	0.471	0.141	0.070	0.100	0.210	1.000			
	r	0.453	0.116	-0.073	0.088	0.178	-0.218	0.198	-0.152	0.000			
Left post CIMT	P	0.773	0.362	0.238	0.189	0.661	0.095	0.758	0.583	0.019	1.000		
	r	0.035	0.111	0.047	0.159	0.053	-0.201	0.038	-0.067	0.281	0.000		
Right ant CIMT	P	0.943	0.068	0.482	0.619	0.740	0.086	0.462	0.423	0.006	0.032	1.000	
	r	0.009	0.219	0.085	0.061	0.040	-0.207	0.089	-0.097	0.327	0.256	0.000	
Right post CIMT	P	0.034	0.150	0.084	0.208	0.226	0.094	0.272	0.951	<0.001	0.004	0.613	1.000
	r	0.253	0.174	0.208	0.152	0.147	-0.202	0.133	-0.008	0.461	0.343	0.061	0.000

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CIMT = carotid intima-media thickness; HsCRP = high sensitivity C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; PASI = Psoriasis Area and Severity Index; SD = standard deviation; TMAO = trimethylamine n-oxide.

Table 3. Correlations between trimethylamine n-oxide and carotid intima-media thickness levels when age, body mass index, disease duration, Psoriasis Area and Severity Index score, blood cholesterol, high sensitivity C-reactive protein and oxidized- low density lipoprotein were controlled.

		TMAO	Left ant CIMT	Left post CIMT	Right ant CIMT	Right post CIMT
TMAO	P	1.000				
	r	0.000				
Left ant CIMT	P	<0.001	1.000			
	r	0.447	0.000			
Left post CIMT	P	0.711	0.028	1.000		
	r	0.048	0.277	0.000		
Right ant CIMT	P	0.905	0.004	0.077	1.000	
	r	-0.015	0.358	0.225	0.000	
Right post CIMT	P	0.047	<0.001	0.025	0.870	1.000
	r	0.251	0.439	0.282	0.021	0.000

CIMT = carotid intima-media thickness; TMAO = trimethylamine n-oxide.

Table 4. Simple linear regression model, predictor: trimethylamine n-oxide, dependent: left anterior and right posterior carotid intima-media thickness.

	R^2	Standardized coefficients beta	t	P	f
Predictor: TMAO Dependent: Left Ant CIMT	0.102	0.320	2.843	0.006	8.084
Predictor: TMAO Dependent: Right Post CIMT	0.020	0.141	1.199	0.235	1.199

CIMT = carotid intima-media thickness; TMAO = trimethylamine n-oxide.

Table 5. Multiple linear regression model adjusted for trimethylamine n-oxide, Psoriasis Area and Severity Index, disease duration, age, ox- low density lipoprotein, total cholesterol, triglyceride, body mass index (dependent variable: left anterior carotid intima-media thickness).

	Standardized coefficients beta	t	P	Variance inflation factor (VIF)
TMAO	0.371	3,801	<0.001	1.169
Age	0.618	5.428	<0.001	1.590
Oxidized-LDL	0.047	0.499	0.620	1.108
HsCRP	-0.040	-0.399	0.691	1.248
Triglyceride	0.078	0.705	0.483	1.503
Total cholesterol	-0.023	-0.209	0.835	1.543
PASI	-0.088	-0.943	0.350	1.068
Disease duration	-0.005	-0.047	0.963	1.420
BMI	-0.079	-0.794	0.430	1.201

BMI = body mass index; HsCRP = high sensitivity C-reactive protein; LDL = low density lipoprotein; PASI = Psoriasis Area and Severity Index

TMAO is a metabolite originating from the gut microbiota that increases the risk of cardiometabolic disease. In regression models, TMAO levels were found to be a positive predictor of cardiovascular disease risk (Figure 4).

CRP is a circulating acute phase reactant that reflects active systemic inflammation. Increased hs-CRP levels are associated with a higher risk of cardiovascular disease, even in individuals without clinical manifestations of atherosclerotic disease [25]. In psoriasis patients, hs-CRP levels are higher than in the healthy population and correlated with the risk of atherosclerotic disease [26,27].

In this study, hs-CRP levels were found to be higher in the patient group compared to healthy controls. In addition, positive correlation between hs-CRP and triglyceride levels
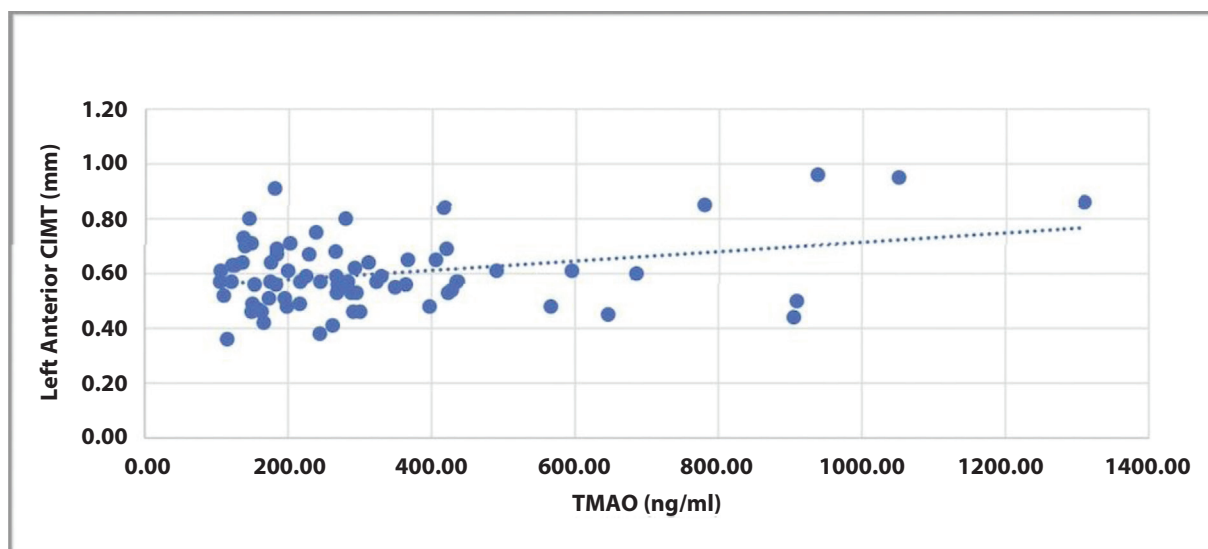
Figure 4. Relationship between trimethylamine n-oxide and left anterior carotid intima-media thickness.

and BMI, negative correlation between hs-CRP and HDL-C in psoriasis patients shows that obesity and metabolic syndrome actually cause systemic inflammatory response.

Cytokine profile observed in psoriasis patients contributes to the formation of dyslipidemia and atherosclerosis through mechanisms such as irregularities in membrane protein synthesis of lipoproteins in the liver, impaired reverse cholesterol transport via HDL-C, the effect of chylomicron residues on atherosclerosis and paradoxical LDL-C increase [28].

In this study, oxidized-LDL and triglyceride levels were higher and HDL-C levels were lower in the patient group compared to healthy controls. There was no statistically difference between the two groups in terms of total cholesterol and LDL-C.

Limitations of the study: this was a cross-sectional study and was conducted in a limited population.

Psoriasis is a chronic systemic inflammatory disease and has been associated with an increased risk of cardiovascular mortality and morbidity in many studies. The data obtained in this study also showed that psoriasis is a risk factor for cardiovascular disease. Although TMAO has been recognized in recent years as an intestinal microbiota and diet-related molecule that triggers atherosclerosis in the vessel wall, there are conflicting results in studies. In this study, serum TMAO levels were significantly higher in psoriasis patients, indicating intestinal dysbiosis, in addition TMAO level is an independent positive predictor for cardiovascular disease risk status. Further studies are needed to determine the diversity of gut bacterial colonization in psoriasis patients, genetic factors, molecular mechanisms controlling gut wall permeability, and the role of probiotics, prebiotics, and perhaps antibiotics to reduce cardiovascular disease risk.

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