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Original Article

Association of TMAO levels with indicators of ulcerative colitis activity

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Abstract

Background: The diagnosis of ulcerative colitis (UC) today is limited to a small number of biomarkers. Trimethylamine-N-oxide (TMAO) is the product of reactions resulting from the degradation of dietary-free choline, phosphatidylcholine, and carnitine metabolism by the intestinal microbiota. Earlier studies showed his involvement in the pathogenesis of UC. To study the association of TMAO with clinical, laboratory, and endoscopic indicators of UC activity.

Methods: an observational cross-sectional comparative study was conducted based on the NCJSC "KMU" clinic, Karaganda, Kazakhstan. High-performance liquid chromatography measured TMAO concentration in 63 patients with UC (age Me 37 (30-52) and 38 healthy individuals (age Me 38 (28.5-49.5).

Results: Median TMAO level in patients with UC-0.286 μmol/l was significantly lower than in the control group Me 0.646 μmol/l ($p<0.0001$). TMAO had significant differences in groups with clinically active and inactive colitis (P=0.003). TMAO correlated with disease activity by Montreal scale (r=-0.389, P=0.002) and severity of attack by Truelove-Witts (r=-0.301, P=0.027 respectively), patient's age (r=0.377, P=0.003), stool frequency (r=-0.427, P=0.001); laboratory parameters: WBC (r=-0.31, P=0.042), blood albumin (r=0.379, P=0.002) and fecal calprotectin (r=-0.314, P=0.022). TMAO did not differ between groups divided by the extent of the pathological process and endoscopic activity.

Conclusion: in patients with UC, TMAO levels decrease compared with healthy individuals and differences in groups depend on the disease activity. These results give reason to consider changes in TMAO levels as a potential marker of UC and the severity of its course.

Keywords: IBD; Inflammatory bowel diseases, Ulcerative colitis, Trimethylamine N-oxide, TMAO, Biomarkers.

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Ulcerative colitis (UC) which is a group of inflammatory bowel diseases (IBD), remains one of the serious problems in modern gastroenterology. Over the past decades, there has been a worldwide trend of increasing incidence of UC in different regions and countries. According to the latest estimates, more than 5 million people suffer from UC worldwide and the number of new cases is increasing every year (1). The severity of course, complications, and mortality, affecting predominantly young and middle-aged individuals, determine the significant public health burden of IBD (2). The main goal in the therapy of UC to avoid the development of adverse outcomes, and early disability and improve the quality of life of patients is endoscopic healing (3). Nevertheless, monitoring laboratory activity allows to assess the efficacy of therapy and to diagnose exacerbation and prevent disease progression in advance of the onset of bloody diarrhea and abdominal pain (3, 4).

An additional advantage of laboratory biomarkers is their non-invasiveness. Currently, C-reactive protein (CRP) and fecal calprotectin (FC), a marker of intestinal inflammation, are traditionally used as laboratory indicators of UC severity (3, 4). However, an increase in CRP is not specific and may reflect an inflammatory process of any localization (5, 6). The use of fecal calprotectin also has limitations, primarily related to difficulties in the assay collection and its instability in samples (7). In addition, fecal calprotectin can also be elevated in other diseases that are accompanied by diarrhea, such as pancreatitis (8). The diagnosis of ulcerative colitis is considered to have an FC level above 150 mcg/g (9). This speaks in favor of the need to search for new biomarkers of UC. Despite the lack of data on the etiology of inflammatory bowel diseases and ulcerative colitis, the study of pathogenesis does not stand still. Along with genetic, and external factors, the influence of changes in the intestinal gut microbiome is widely studied (10-12). It has been suggested that the pathogenesis of ulcerative colitis may be due to altered and pathogenic interactions between the host immune system and its commensal intestinal microbiota. It was previously revealed that the so-called Nod-like (NLRP3) receptors of the pyrin domain family of inflammasome 3 receptors determine the severity of inflammation in the intestinal wall and their dysregulation may contribute to the development of ulcerative colitis (13-15). Also, an important aspect is that disturbances in the composition of the commensal intestinal microbiota entail changes in the content of metabolites produced by it. Active study of this issue has led to speculation about the involvement of one of the metabolites, trimethylamine-N-oxide (TMAO), in the pathogenesis of ulcerative colitis.

TMAO is the final product of reactions resulting from the degradation of dietary free choline, phosphatidylcholine, and carnitine metabolism by the intestinal microbiota to form the metabolite precursor trimethylamine and its N-oxidation by the hepatic enzyme flavin-containing monooxygenase isoform 3 (FMO3) in the liver (16-19). The final concentration of TMAO formed depends on each step of this metabolism. Chao et al. were the first to suggest the involvement of TMAO in the pathogenesis of inflammatory bowel diseases. They found that TMAO significantly inhibits the expression of ATG16L1, LC3-II, and p62 and triggers NLRP3-activated inflammasome with the production of reactive oxygen species in human colon cells, contributing to the development of intestinal wall damage (20). Much attention is also paid to the nature of the patients' nutrition. In a systematic review in 2020, Li assessed the risk of developing CDD when adhering to a “Western-style diet”. This diet involves eating large quantities of meat, animal protein, and dairy products, while reducing the intake of plant foods, vegetables and fruits. In the paper, they demonstrated that the “Western type” of diet was associated with the risk of developing UC (RR 2.15, 95% CI 1.38-3.34) (21). There are sporadic works where TMAO is considered a biomarker of IBD. In the study by Wilson A. et al., it was found that in patients with IBD, the level of TMAO is lower than in healthy individuals and has significant differences between patients with active and inactive colitis by clinical evaluation (22). In 2023, Banno Yukika et al.published the results of a “Mendelian randomization study” that examined the risks of developing IBD with low TMAO levels. They found that the odds ratio for UC per 1 interquartile range increment (per 2.4 μmol/L) in TMAO levels was 0.88 and was not significant (0.76-1.02, P = 0.089) for ulcerative colitis. In the article, the authors emphasized that this issue is of great interest and that more research is needed (23). Studying the relationship between inflammatory biomarkers, TMAO, and endothelial dysfunction in patients with IBD, Seref et al. found that the level of TMAO was lower in patients with IBD compared to controls. However, it was statistically not significant (24). There are no works on the evaluation of TMAO levels in patients with UC depending on the degree of inflammation activity. Our study aimed to investigate the associations of TMAO level with clinical, laboratory, and endoscopic indicators of ulcerative colitis activity.

Methods

Design: The presented observational cross-sectional comparative study was conducted at the Medical University Clinic of the Karaganda Medical University from September 2022 to December 2023. The participants of the study were 101 people, 63 patients with UC and 38 healthy people of the control group. The minimal age of the study participants was 19 years, and the maximum age was 72 years. Urban residents prevailed over rural residents: 78.8% vs. 21.2%, respectively. The selection was carried out according to inclusion and exclusion criteria. Patients with UC were mandatory: age over 18 years; residence in Karaganda region (Central Kazakhstan region); confirmed diagnosis of ulcerative colitis according to clinical, laboratory, endoscopic and histologic data with various degrees of activity according to international classifications (24); no previous antibiotic therapy for the last 6 months; no intake of NSAIDs and probiotics for 1 month before stool sampling. According to the clinical guideline of the Ministry of Health Republic of Kazakhstan “Ulcerative

Colitis" of 2017, all patients use the recommended low-FODMAP diet, which has no limitation on red meat, eggs, or fish. During the patient survey, nutrition was assessed including caloric intake, energy value and the average content of foods rich in choline and carnitine in the diet. Patients with UC were not limited in foods that were a source of choline and carnitine and did not differ from healthy individuals. The control group consisted of respondents over 18 years of age, with no history of chronic pathologies and no previous antibiotic therapy for acute diseases in the last 6 months, residents in Karaganda region. The gender and ethnicity of participants were not a selection criterion. From the study, the excluded persons are those with psychiatric, severe neurological diseases, pregnant and lactating women and persons who refused to participate in the study.

The sample size was calculated using the EpiINFO calculator, based on epidemiologic data on the population of Karaganda region over 18 years of age at the time of the study and the morbidity of UC in the region. Each participant was acquainted with the goals, objectives, and methods of this study and signed an informed consent form before being included in the study. After consent was obtained clinical examination and blood and fecal samples were collected. Standard operating procedures were developed to implement the process of subject recruitment, protection of participants, collection, transportation, and storage of biological samples. The study protocol, informed consent form of the participant and standard operating procedures were approved at the Local Bioethics Commission of NCJSC "Medical University of Karaganda" meeting. Minutes No. 1 of 20.09.2022 of the meeting of the Local Bioethics Commission of the Karaganda Medical University.

Data collection: The clinical study of the participants consisted of an interview with the collection of data on the course of the disease, physical examination and analysis of laboratory, instrumental and histological studies, which were subsequently formed in a unified MS Excel table (passport, anthropometric data; features of the history of the disease, including diagnosis and its course; clinical manifestations; extent and activity of the inflammatory process; characteristics of treatment; results of laboratory tests). To detect the presence of undernutrition and assess the risk of developing undernutrition, we used NRS-2002 scale (26). Patients with ulcerative colitis were questioned using the Simple Clinical Colitis Activity Index (SCCAI) questionnaire. According to the international classifications used to characterize ulcerative colitis, the main group's patients were divided depending on the activity, severity of attack and extent of the pathological process (27, 28). The SCCAI clinical index was defined by the sum of scores on the evaluation from 0 to 3 of the following characteristics: Bowel frequency at day/night, Urgency of defecation, Blood in stool, General health, and Extracolonic manifestations. A score of less than 2 was considered inactive colitis. Montreal activity stage was assessed based on the number of defecations per day, presence, and volume of blood in the stool, pulse rate, body temperature, hemoglobin level and erythrocyte sedimentation rate (ESR). The activity was determined according to 4 groups, where S0-remission, S1-mild degree, S2-moderate degree, and S3-severe degree. Assessment of severity according to Truelove-Witts's criteria consisted of frequency of stools with blood, pulse, body temperature, hemoglobin and ESR. Further patients were divided into groups with mild, moderate, and severe UC attacks.

Laboratory tests: After completion of clinical examination in the procedure room, the participants underwent blood sampling by venipuncture into 3 vacuum tubes with 5 ml volume: 2 tubes containing anticoagulant EDTA (general blood analysis, TMAO) and 1 tube with coagulation activator (biochemical blood analysis) and stool sampling. Biological sampling was performed 1-3 days before endoscopic examination. (Vacuum Blood Collection Tube, Gel&Clot Activator Tube, EDTA Tube, Chengdu PUTH Medical Technology Co.,Ltd., China). Peripheral blood parameters were determined using automated analyzers after preliminary centrifugation of samples. For general blood analysis, we used a high-performance hematological automatic 6-diff closed-type analyzer Sysmex XN-2000 (Japan), biochemical analysis was performed on a closed-type analyzer Beckman Coulter (Japan). Fecal calprotectin concentration was measured by ELISA method using EliA Calprotectin 2 test system Phadia GmBH (Germany). To determine the level of TMAO, venous blood was taken and collected after 12-hour fasting in K2EDTA vacutainers. Whole blood was centrifuged (3000 rpm, 15 minutes) immediately after collection and the resulting plasma was frozen and stored until analysis at -80°C in ultra-low temperature refrigerators. The following reagents were used in the study: trimethylamine N-oxide (95%, Sigma-Aldrich), formic acid (≥95%, Sigma-Aldrich), acetonitrile (≥99.9%, Sigma-Aldrich), highly purified water (18.2 MΩ) obtained using the Milli-Q system (Millipore). For analysis, 100 µl of plasma was used, to which 600 µl of acetonitrile was added, followed by centrifugation with a relative centrifuge acceleration of 20.000 g for 10 minutes, at a centrifuge temperature of 4°C. The supernatant, in an amount of 100 µl was transferred into a vial then 100 µl of

water was added to it. The resulting solution was injected into the HPLC – MS/MS system; the volume of the injected sample was 10 µl. The analysis was performed using an Agilent 1260 Infinity HPLC system and G6130A Quadrupole LC/MS. For separation, a ZORBAX Eclipse XDB 80Å C18, 2.1 x 75 mm, 3.5 µm column was used, a Zorbax Eclipse XDB C-18 protective column, 12.5 x 4.6 mm, was also used; with a particle size of 5 microns. The separation was carried out in an isocratic mode using eluents: A — 0.125% solution of formic acid in acetonitrile, B — 0.125% solution of formic acid in water, in a ratio of 1:1, with a flow rate of 0.250 ml/min. The temperature of the column thermostat was 30°C. The mass spectrometer worked in multiple reaction monitoring (MRM) mode, with an ESI source. The equipment was calibrated using the Agilent Lab Advisor software, system control and data analysis were performed using the Agilent ChemStation software. The quantitative content of TMAO in µmol/l in the analyzed samples was calculated using the external standard method (29).

The endoscopists who performed the study patients had more than 10 years of procedural experience. To avoid errors, the endoscopists were not informed about the clinical course and severity of the disease, fecal calprotectin results, and changes in peripheral blood before the procedure. Endoscopic examination was performed within 1-3 days after collection of blood and fecal samples. The endoscopic activity of colitis was evaluated by the number of scores Ulcerative Colitis Endoscopic Index of Severity (UCEIS) depending on the changes in the vascular pattern, the presence of bleeding, erosions and ulcers. The total score was used as a division into groups, where the number of UCEIS scores from 0 to 1 corresponded to remission, 2-4 points - to minimal activity, 5-6 points - to moderate activity, and 7-8 points - to marked activity (30).

Statistical processing: Statistical processing of data was carried out using the IBM SPSS Statistics 22 program package. The normality of data distribution was checked using the one-sample Kolmogorov-Smirnov criterion. The characteristics of the data having normal distribution were described using the arithmetic mean with standard deviation; in the case of non-normal distribution - median and interquartile range. Comparative analysis of qualitative data was performed using the Chi-square criterion, quantitative data between 2 groups using the Mann–Whitney U test; when comparing several groups, the Kruskal — Wallis test was applied. The nonparametric Spearman test was used to assess correlations. Differences between groups were considered statistically significant at the level of $p \leq 0.05$ (significance level $\alpha=0.05$).

Results

Figure 1 demonstrates the process of patient selection. Out of 323 people suffering from ulcerative colitis and 38 healthy individuals, 101 people were included in the study by exclusion. The main group consisted of 63 patients, a control group of 38 people.

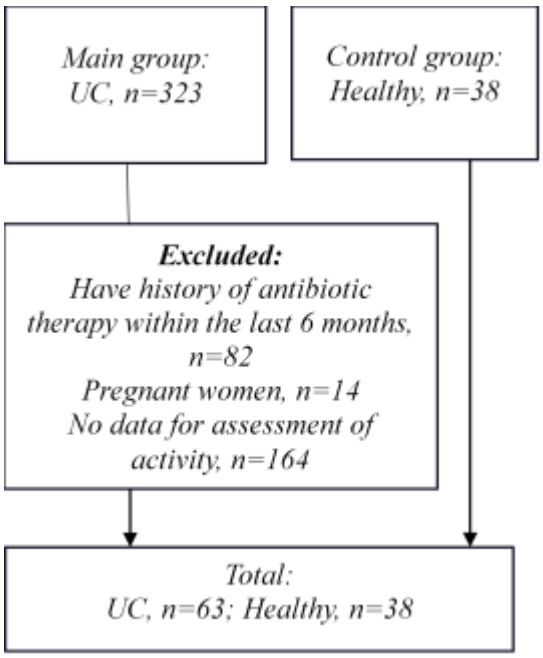

Figure 1. The process of selecting research subjects

Patients' characteristics: Demographic, clinical, and laboratory characteristics of the patients are presented in table 1. The gender ratio of males and females is presented in equal proportion, most of the patients were young. The main and control groups had no significant differences in gender and age. In the main group, prevailed patients with active disease, extensive lesions, and moderately severe stage.

The groups had significant differences in laboratory parameters such as leukocytes, lymphocytes, neutrophils, basophils, ESR, total protein, albumin, CRP, and FC. Patients with ulcerative colitis had higher medians of inflammatory response indicators- leukocytes, neutrophils, ESR, and CRP. At the same time, the indicators of nutritional disorders such as total protein and albumin were lower compared to the group of healthy individuals.

Comparison of TMAO levels between groups (figure 2) showed that TMAO levels were significantly lower in patients with UC (Me 0.286 μmol/L, Q25-Q75=0.11-0.61) compared to the control group (Me 0.646 μmol/L, Q25-Q75=0.46-1.04), $p < 0.001$. A comparison of TMAO levels in the groups of UC patients depending on the activity and extent of the inflammatory process and with the control group is presented in table 2. Evaluation of TMAO in groups divided by clinical activity of colitis according to the SCCAI questionnaire demonstrated a significant difference in TMAO levels in groups with active and inactive colitis (P=0.003). In the group with inactive colitis, the median TMAO level did not differ from the control group. In the group of patients with active colitis, significant differences in TMAO levels were found compared to healthy individuals ($p<0.0001$). Analysis of TMAO levels depending on the activity according to the Montreal scale showed significant differences between the groups ($p=0.016$). Higher values of TMAO level were in patients in remission and with low disease activity. The median TMAO level decreased with increasing activity index. Significant differences ($p<0.0001$) were found in groups with moderate and pronounced activity corresponding to S2, S3 index compared to the control group.

Table 1. Patients' characteristics

Demographic characteristics				
		UC patients (N, %)	Control (N, %)	χ^2, p-level
Total		63	38	
Gender	**Male**	31 (49.2)	18 (47.4)	0.089, 0.756
	Female	32 (50.8)	20 (52.6)	
Occupation	**Urban**	78.8	83	
	Village	21.2	17	
		Me (Q25-Q75)		**p-value**
Age		37 (30-52)	38 (28.5-49.5)	0, 974
Clinical characteristics				
		UC patients (N, %)	**Control (N, %)**	
SCCAI	**Active disease**	51 (81)	-	
	Non-active disease	12 (19)	-	
Extent	**Proctitis**	6 (9.5)	-	
	Left-sided colitis	30 (47.6)		
	Pan-colitis	27 (42.8)	-	
Montreal activity	**S0**	6 (9.5)	-	
	S1	25 (39.7)	-	
	S2	25 (39.7)	-	
	S3	7 (11.1)	-	

Truelove and Witts Severity	**Mild**	18 (36.5)	-
	Moderate	29 (46)	-
	Severe	11 (17.5)	-
UCEIS	**Remission**	14 (22.2)	-
	Minimal	16 (25.4)	-
	Moderate	25 (39.7)	-
	Severe	8 (12.7)	-
Treatment	**5-ASA**	63 (100)	-
	Steroid	16 (25.4)	-
	Cytostatics	3 (4.8)	-
	Biology	6 (9.5)	-
NRS-2002 risk	**0 points**	38	-
	1 point	19	-
	2 point	5	-
	3 points	1	-

Laboratory characteristics	**Me (Q25-Q75)**		**p-value**
	UC patients	**Control group**	
Hb, g/l	132 (115-144)	135 (126-144)	0.143
RBC, x 10^{12}/l	4.58±0.659	4.65 (4.29-4.95)	0.837
WBC, x 10^{9}/l	6.7 (5.2-8.6)	5.5 (4.7-6.9)	0.012
PLT, x 10^{9}/l	286 (230-358)	269 (228-317)	0.279
LYM, %	30 (21.8-36.8)	34.5 (30-41.8)	0.007
NEUT, %	58.1 (21.8-36.8)	52.6 (46-58)	0.039
BAS, %	0.6 (0.4-0.8)	0.8 (0.6-0.9)	0.026
MON, %	7.6 (6.2-8.9)	8 (7.2-8.9)	0.209
EOS, %	2.5 (1.1-4)	2.6 (1.5-3.1)	0.961
ESR, mm/h	13 (7.5-26)	8 (2-11)	0.002
Total protein, g/l	70 (63-73)	73.7 (69.5-75.7)	0.007
Albumin, g/l	41.4 (37.8-44.4)	45.7 (44-49)	< 0.001
CRP, g/l	2.7 (1-8.3)	0.9 (0.5-2.2)	< 0.001
Fecal calprotectin, mcg/g	296.4 (63.11-1292)	19.5 (11-27)	< 0.001

5-ASA-5-aminosalicylic acid, Hb-Hemoglobin, RBC-Red blood cells, WBC-white blood cells, PLT-platelets, LYM-lymphocytes, NEUT-neutrophils, BAS-basophils, MON-monocytes, EOS-eosinophils, ESR-erythrocyte sedimentation rate, CRP-C-reactive protein.

Table 2. Comparison of TMAO levels in groups of UC patients depending on the activity and extent of the inflammatory process and with the control group

	Me (Q25-Q75) TMAO, µmol/l	p_1	p_2
SCCAI activity			
Non-active colitis	0.896 (0.208-1.263)	p=0.003***	0.727
Active colitis	0.241 (0.111-0.48)		<0.0001
Montreal activity			
Clinical remission	0.425 (0.146-1.303)	p=0.016	0.44
Mild UC	0.447 (0.157-1.153)		0.119
Moderate UC	0.234 (0.109-0.472)		<0.0001
Severe US	0.124 (0.015-0.243)		<0.0001
Truelove-Witts activity.			
Mild	0.411 (0.135-1.02)	p=0.048	0.053
Moderate	0.311 (0.11-0.611)		0.003
Severe	0.196 (0.024-0.264)		<0.0001
Extent			
Proctitis	0.199 (0.145-1.14)	p=0.386	0.149
Left-sided colitis	0.408 (0.167-0.613)		0.008
Extensive/total	0.264 (0.099-0.516)		<0.0001

*p1- p-level of the Kruskal — Wallis one-way analysis (comparison of TMAO levels in groups of UC patients depending on the activity and extent of the inflammatory process).
**p2 - p-level of Mann-Whitney U Test (reliability of differences of TMAO level indices in UC patients in comparison with the control group). ***p- p-level of Mann-Whitney U Test.

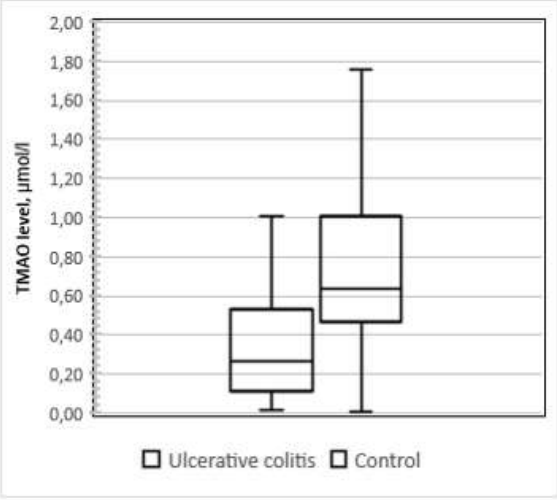

Figure 2. TMAO level in patients with IBD and Control (Healthy)

When comparing TMAO concentrations in patients with low When comparing TMAO concentrations in patients with low, moderate, and severe attack activity according to the Truelove-Witts scale, statistically significant differences between the groups were also found (P=0.048). TMAO level decreased with increasing severity of UC attack. Significant differences in TMAO levels were found in patients with moderate and severe degrees of UC activity compared to the control group. It was found that the level of TMAO in the groups divided by the extent of pathologic process in the large intestine had no significant differences (P=0.386). TMAO levels differed compared to the control group in patients with left-sided (P=0.008) and total colitis (p<0.0001). It is known that a level of FC more than 150 μg/g is considered a significant threshold for identifying active ulcerative colitis (9). We divided patients into groups according to the presence of activity with fecal calprotectin concentration of more than 150 mg/kg and with no laboratory activity with fecal calprotectin of less than 150 mg/kg. The following parameters were significantly different between these groups: age (P=0.02), hemoglobin (p=0.044), leukocytes (P=0.047), ESR (P=0.043), albumin (P=0.005), CRP (P=0.015), fecal calprotectin (p<0.0001), and the sum of endoscopic activity scores (P=0.001).TMAO levels differed according to fecal calprotectin value in groups with less and more than 150 mg/kg (p=0.005). The median for inactive UC as measured by FC was Me 0.450 (Q25-Q75=0.218-0.899) and for active UC Me 0.241 (Q25-Q75=0.109-0.513).

Correlation of TMAO level with the activity of the process and its indicators: Correlation analysis of the relationship between TMAO level and various indicators (table 3) characterizing the activity of the inflammatory process showed that TMAO increased with increasing age of the patients (r=0.377, P=0.003). According to clinical indicators, TMAO had an inverse correlation with stool frequency, the more pronounced diarrhea in the patient, the lower was TMAO index (r=-0.427, P=0.001). The correlation was also established by the disease activity scale (Montreal) and severity of attack (Truelove-Witts). With an increasing index of colitis activity and more severe attack, TMAO decreased (r=-0.389, P=0.002 and r=-0.301, P=0.027 respectively).

In the context of blood laboratory parameters, we obtained data on the presence of a negative correlation relationship between TMAO content and peripheral blood leukocyte count (r=-0.31, P=0.042). The level of TMAO had a positive correlation with the level of blood albumin (r=0.379, P=0.002). Marker of intestinal inflammation-fecal calprotectin was negatively correlated with TMAO (r=-0.314, P=0.022). There was no significant relationship between the extent of the pathological process, and peripheral blood parameters such as hemoglobin, erythrocytes, platelets, ESR, total protein, CRP. We have established significant correlations with the indices of clinical and laboratory activity of UC, but no significant correlation of TMAO level with the index of endoscopic activity was found. Meanwhile, the study of the relationship of fecal calprotectin also demonstrated a more pronounced relationship with the severity of the course of colitis according to the Montreal scale (r =0.472, p=< 0.0001) and attack according to the Truelove-Witts scale (r =0.540, p=< 0.0001) compared to the endoscopic activity index (r =0.309, P=0.015) and correlation with lesion extent (r =0.373, P=0.03). As identified previously, fecal calprotectin itself has a significant relationship with TMAO levels.

Table 3. Evaluation of the relationship of TMAO with activity indices, laboratory parameters and age of patients in ulcerative colitis

	r (Spearman)	p-level		r (Spearman)	p-level
Age	0.377	0.003	**WBC**	-0.31	0.402
Gender	-0.86	0.5	**PLT**	-0.054	0.697
Frequency of defecation	-0.427	0.001	**LYM**	0.216	0.094
Extension	-0.147	0.254	**NEUT**	-0.168	0.196
Truelove-Witts	-0.301	0.027	**ESR**	0.199	0.124
Montreal	-0.398	0.002	**Total protein**	0.222	0.086
UCEIS	0.129	0.349	**Albumin**	0.397	0.002
Hb	0.046	0.725	**CRP**	-0.053	0.684
RBC	0.092	0.483	**Fecal calprotectin**	-0.341	0.022

UCEIS-The Ulcerative Colitis Endoscopic Index of Severity, Hb-Hemoglobin, RBC-Red blood cells, WBC-white blood cells, PLT-platelets, LYM-lymphocytes, NEUT-neutrophils, ESR-erythrocyte sedimentation rate, CRP-C-reactive protein.

Discussion

In this study, we examined the association of TMAO levels with clinical, laboratory, and endoscopic indicators of ulcerative colitis activity. We have shown for the first time that the level of TMAO significantly differs in patients depending on the clinical and laboratory activity according to the Montreal index and the severity of the attack during the exacerbation of the disease according to the Truelove-Witts scale, the relationship between the level of TMAO and fecal calprotectin was studied and established. The possibility of using TMAO as a biomarker for IBD was investigated by Wilson, Teft, and Morse et al. The results of the study demonstrated that in comparison with healthy individuals, TMAO in the group with IBD was lower. When comparing TMAO levels according to disease activity, it was found that patients with ulcerative colitis in the exacerbation stage had lower levels of TMAO; there were no differences in the group with Crohn's disease. Disease activity in this study was assessed based on indices (Simple Clinical Colitis Index for individuals with UC and the Harvey-Bradshaw Index for individuals with Crohn's Disease), including only clinical manifestations - stool frequency, blood in the stool, urgency of defecation, general well-being, extraintestinal manifestations (22). Subsequently, Seref et al. in evaluating the relationship of TMAO and inflammatory biomarkers with endothelial and coronary microvascular dysfunction in 56 patients with inactive IBD, found that TMAO levels were lower in IBD patients compared to controls, although not statistically significant (24). In our study, we confirmed these results by finding that TMAO levels were significantly lower in patients with IBD compared to controls. We also obtained the results of differences in TMAO levels between patients with active and inactive colitis by clinical evaluation and no significant differences between TMAO levels in patients with inactive UC and controls. It is worth noting that there are no reports in the sources on the study of TMAO in groups that are divided according to the degree of activity and severity of ulcerative colitis attack. We found that TMAO levels differed significantly according to the activity of the course of colitis as assessed by the Montreal scale and the severity of attack according to the True-love-Witts scale. Patients with more severe ulcerative colitis had lower TMAO levels than patients with low activity and in remission.

We did not find a significant association of TMAO with CRP levels, the presence of which was previously shown in a study by Seref et al. (24). This may be due to the prevalence of patients with moderate to mild activity in our study. We obtained evidence of a direct correlation between TMAO concentration and albumin levels, which in patients with IBD may be due to both continuing diarrhea and dietary changes. It should be noted that for the first time, we established the presence of a significant negative correlation with fecal calprotectin, which according to many studies (31-33) is a marker of clinical and endoscopic activity of ulcerative colitis. We did not find a significant relationship between TMAO levels and endoscopic activity.

Possible reasons for the decrease in TMAO levels in patients with ulcerative colitis may include reduced intake of foods containing choline, carnitine and phosphatidylcholine, as well as the genotype of the enzyme responsible for the conversion of TMA to TMAO in the liver, flavin monooxygenase.

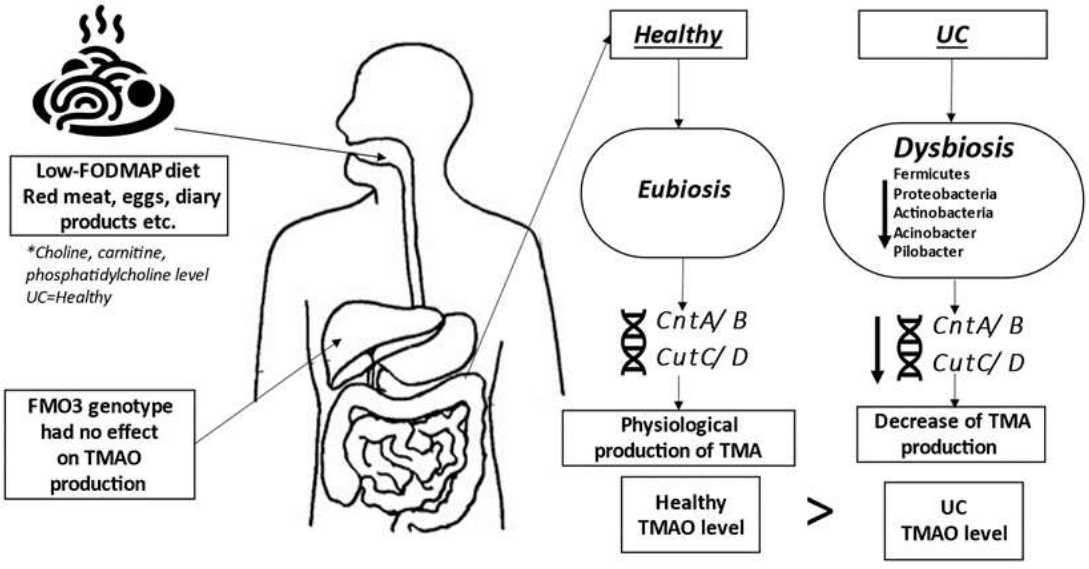

Figure 3. The process of TMAO formation in IBD. Adapted from Wilson et al. (22, 34-36)

Wilson et al. showed that the decrease in TMAO levels in UC patients compared to healthy individuals was not accompanied by a significant difference in choline and carnitine content and did not depend on the genotype of flavin monooxygenase 3 enzymes. As noted earlier, adherence to the low FODMAP diet did not affect differences in choline and carnitine levels between healthy individuals and patients with UC. Earlier it was emphasized that in the mechanism of IBD and UC development a special role is given to the disturbance of the host microbiome, which undoubtedly affects its ability to produce various metabolites, including TMAO. His precursor trimethylamine- can be produced by "choline and carnitine-consuming bacteria" from the families Firmicutes, Proteobacteria and Actinobacteria (34). Currently, based on microbiome sequence models, several pathways of bacterial TMA formation such as choline-TMA lyase CutC, carnitine monooxygenase, CntAB, and glycine-betaine reductase of bacterial strains (35). Even though the intestinal microbiota plays an important role in this process, only a few of its representatives have genes containing the anaerobic glycyl radical choline-TMA-lyase CutC/D and aerobic carnitine monooxygenase CntA, which contribute to the formation of TMA from choline and carnitine (36). The identification of these enzymes with the subsequent use of full genome sequencing allowed us to suggest that the increase in TMAO may be promoted by the increased content of representatives of the genus *Acinetobacter (e.g., Acinetobacter Calcoaceticus, Acinetobacter baumannii)* due to the presence of the CntA/B gene and *Pelobacter (e.g., Pelobacter acetylenicus, Pelobacter carbinolicus)* having CutC/D genes (37).

Thus, changes in the composition of the microbiome affect the amount of TMA produced and, accordingly, the further formation of TMAO, and in the development of dysbiosis accompanied by a decrease in the number of the above-described families, the concentration of TMAO will logically decrease. It is also important that changes in the microbiome trigger a cascade of its pathogenic interactions with the immune system, affecting both the development of ulcerative colitis and the severity of the course (13, 14). Another cause of decreased TMAO, which has been established in both laboratory animals and patients, was the administration of broad-spectrum antibiotics for 3-4 weeks (38, 39). In our study, patients taking antibiotics within the last 6 months were excluded. Considering the metabolic "pathway" of TMAO formation, assumed that the reason for the decrease in TMAO levels is primarily changes in the composition and imbalance of the intestinal microbiome with increasing activity of ulcerative colitis. The explorations suggest that TMAO levels reflect changes in the microbiome in ulcerative colitis. Further studies are needed to understand better the mechanism of TMAO participation in the mechanism of ulcerative colitis development, and its relationship with microbiome changes.

The results of our study show that TMAO is decreased in patients with ulcerative colitis and varies with the severity of the disease. We obtained data that TMAO decreases in case of active colitis by clinical and laboratory parameters, depends on the patient's age. It is suggested that the determination of TMAO levels may be a useful non-invasive tool for diagnosing ulcerative colitis, determining the severity and monitoring the disease and give reason to consider changes in TMAO levels as a potential marker of UC and the severity of its course. These findings allow us to expand our research to investigate the issue of microbiome alteration, its relationship with TMAO, and the use of TMAO as a marker for microflora correction in patients with ulcerative colitis.

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