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ORIGINAL ARTICLE

The Gut Microbe-Derived Metabolite Trimethylamine N-Oxide in Patients With Systemic Lupus Erythematosus

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Abstract

Background/Aim: Both human and animal studies suggest that the gut microbe-derived metabolite trimethylamine N-oxide (TMAO) is strongly associated with several autoimmune diseases including systemic lupus erythematosus (SLE) and correlates to disease severity. The study aimed to investigate the diagnostic and prognostic validity of TMAO as a potential biomarker in patients with SLE, particularly focusing on lupus nephritis patients and its relation to disease activity.

Methods: A total of 90 patients were included and assigned into either: group I (SLE without nephritis (NN)), group II (lupus nephritis (LN)) and group III (healthy controls). Serum TMAO levels were compared between the study groups and correlated to the clinical, laboratory and histopathological criteria.

Results: Unpredictably, TMAO levels were significantly higher in healthy controls compared to the total SLE population ($p = 0.003$), to LN and NN groups individually ($p = 0.01$). TMAO levels did not significantly vary between (NN) and (LN) patients and only correlated to anti-dsDNA titres ($p = 0.02$) and red blood cells count ($p = 0.02$) among LN patients.

Conclusion: Contrary to previous studies, TMAO levels were found to be higher in healthy controls. A possible confounding effect of the dietary pattern and ingested drugs on the gut microbiome limits the utility of TMAO as a potential marker in different diseases.

Key words: Trimethylamine N-oxide (TMAO); Systemic lupus erythematosus; Lupus nephritis; Gut microbiome; Disease activity.

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that is characterised by the presence of hyperactive immune cells and aberrant antibody responses to nuclear and cytoplasmic antigens.[1] The "hygiene hypothesis" is a popular hypothesis accounting for the role of the gut microbiome in the pathogenesis of SLE. The hygiene hypothesis states that as incidence of bacterial infection have decreased with increased hygiene standards and practices, both pathogenic and non-pathogenic colonisation of the gut have also decreased.[2] The earliest evidence for a potential role for intestinal microbiota in SLE came from a report by Gul'neva et al in 2007 who described genera that dominated the intestinal microbiota of SLE patients.[3] Later reports revealed that SLE patients had a reduced bacterial diversity in their faecal samples, compared with individuals from the general population.[4]

SLE patients demonstrate a decrease in Firmicutes levels, exhibiting a 2.5-fold decrease in the

ratio of Firmicutes to Bacteroidetes.[1] Butyrate, produced by Firmicutes, plays a role in the differentiation of regulatory T cells in the colon, spleen and lymphatic system that suppress inflammation, the dysfunction of which is a hallmark of SLE and a precursor to cardiovascular incidents.[5] Therefore, a decrease in Firmicutes and consequent decrease in butyrate levels may contribute to inflammation in SLE patients.[6] This hypothesis is further supported by evidence demonstrating that antibiotics, which diminish populations of butyrate-producing Firmicutes such as trimethoprim–sulfamethoxazole, minocycline and amoxicillin have been shown to trigger lupus flares.[2] It is worth noting that *Lachnospiraceae*, butyrate-producing bacterium of the Firmicutes phylum are present in higher numbers in SLE patients than in their healthy counterparts. Therefore, it is possible that *Lachnospiraceae*, or any butyrate-producing bacteria, may not be able to suppress inflammation in SLE cases.[6]

The relative increase in Bacteroidetes in the SLE microbiome results in heightened Toll like receptor 4 (TLR-4) activity that has been associated with spontaneous lupus development.[7, 8] Adjustment of the SLE microbiome *via* dietary intervention has shown attenuation of SLE symptoms. The ingestion of retinoic acid, a metabolite of vitamin A as a dietary intervention restored normal *Lactobacilli* levels in lupus-prone mice.[6] Vitamin A ingestion has also been shown in a very small study to ameliorate lupus nephritis and proteinuria.[9] Finally, increases in *Lactobacilli* in SLE murine models have been shown to suppress pro-inflammatory responses.[6]

Recent advances in "metabolomics" have broadened insights of the metabolites produced or metabolised by the gut microbes, which can serve as important immune regulators or initiators in a wide variety of diseases, including autoimmune diseases.[10] However, there is a scarcity of studies investigating the metabolites of altered gut microbiota in SLE patients where TMA/TMAO have been studied.[11, 12] In humans, trimethylamine (TMA) is synthesised exclusively by gut microbiota from dietary nutrients contained in high-fat foods including choline, L-carnitine and other TMA-containing nutrients. TMA produced in the gut enters the circulation and is further converted into trimethylamine N-oxide (TMAO) by host enzymes like flavin monooxygenase (FMO) in the liver.[13, 14]

Eight species of human commensal bacteria were identified to produce TMA from choline including two different Firmicutes.[15] Both human and animal studies suggest that the gut microbe-derived metabolite TMAO is strongly associated with cardiovascular disease.[16] TMAO has been positively linked to the pathogenesis of several other diseases as well, including chronic kidney disease, type 2 diabetes and obesity in a tissue- and cell-specific pattern.[17-20] Direct exposure to TMAO can augment Ca^{2+} release, enhance platelet activation and form pro-thrombotic state.[21] TMAO activates mitogen-activated protein kinase (MAPK) in vascular endothelial cells and smooth muscle cells, which promotes the expression of inflammatory genes and recruitment of activated leukocytes.[22] Dietary choline and TMAO initiate renal fibrosis and dysfunction in animal models *via* a transforming growth factor-β (TGFβ)–phospho-SMAD3 (P-SMAD3) signalling pathway.[19] The metabolite perturbations related to rheumatic disease remains poorly resolved and merits further studies.

The aim of this study was to investigate the diagnostic and prognostic validity of TMAO as a potential biomarker in lupus nephritis patients and its relation to disease activity.

Methods

Study sample

This was a cross sectional study conducted to assess the role of TMAO as a potential biomarker in patients with SLE, conducted for 1-year duration. The study included 90 subjects who were classified into 3 groups: Group (I): included patients who were diagnosed as SLE without nephritis (NN); Group (II): included patients who were diagnosed as lupus nephritis (LN) and Group (III): included healthy subjects as a control. Male and female SLE patients diagnosed according to 2019 European League Against Rheumatism and American College of Rheumatology classification criteria for SLE[23] and aged > 18 years with willingness to participate in the study were included. Patients who refused the enrolment in the study, patients with overlap syndrome, patients with organ specific or other systemic autoimmune disorders, patients with non-SLE renal affection eg diabetic or hypertensive nephropathy and primary or other

secondary glomerulopathies and pregnant SLE female patients were excluded from the study.

Data collection

The medical records of patients were reviewed using a computerised sheet including all studied data for each patient. The authors have followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. All patients were subjected to the following: complete history taking; thorough clinical examination; SLE international Collaborating Clinics/ American College of Rheumatology Damage Index (SLICC/ACR); Systemic Lupus Erythematosus Disease Activity Index (SLEDAI); laboratory investigations including: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), kidney function test, urine analysis, 24-hour urinary protein, antinuclear antibody (ANA), complement (C3, C4) and anti-ds DNA; kidney biopsy; radiology - abdominal sonography with emphasising on kidneys.

The SLICC/ACR Damage Index (SDI) was developed in 1996 to assess an ongoing reflection of disease activity in SLE patients and to measure irreversible damage resulting from SLE disease activity and its treatment.[24] SLEDAI index is composed of 24 features that are attributed to lupus which are listed, with a weighted score. The more serious manifestations (such as renal, neurologic and vasculitis) are weighted more than others (such as cutaneous manifestations). The maximum possible score was 105.[25] Kidney biopsy was performed for all patients fulfilling LN ACR criteria to confirm the diagnosis and to classify the glomerular disease by current International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification.

Renal histopathological examination

The renal histopathology was evaluated according to the WHO classification of LN with assessment of activity and chronicity indices as follows: activity index (AI). This index was assessed as the sum of individual scores of the following items considered to represent measures of active lupus nephritis: glomerular proliferation, leucocyte exudation, karyorrhexis/fibrinoid necrosis (x2), cellular crescents (x2), hyaline deposits and interstitial inflammation. The maximum score was 24 points for the AI.[26] Chronicity index (CI) consisted of the sum of individual scores of the following items considered to represent measures of chronic irreversible lupus nephritis: glomerular sclerosis, fibrous crescents, tubular atrophy and interstitial fibrosis. The maximum score was 12 points for the CI.[27]

Sampling

Urine and venous blood samples were obtained after overnight fasting. Trimethylamine levels were measured using the quantitative sandwich enzyme linked immunosorbent assay method, with Human (ELISA) kit (Cat No E4733Hu). The plate has been pre-coated with human TMAO antibody. The standard curve was constructed by plotting the average optical density (OD) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph. These calculations were performed with computer-based curve-fitting software.

Statistical analysis

Data were coded, computed then analysed using Statistical package for social science (IBM SPSS) version 24 for Windows. Qualitative data were presented by frequency tables. For quantitative variables the normality of data was first tested with Shapiro-Wilk test and presented data by central indices and dispersion: mean ± standard deviation (SD) for normally distributed variables and median (minimum – maximum) for non-normally distributed variables. Chi-square test was used to test association between categorical variables. It was replaced by Fisher Exact Test if the expected cell count was less than 5 in four-cells tables, while it was replaced by Monte Carlo test if the expected cell count was less than 5 in more than four-cells tables. Association between normally distributed continuous variables was tested using independent sample t-test in 2 independent groups, while Mann-Whitney U test (z) was used to compare two independent nonnormally distributed continuous variables. The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of two or more independent (unrelated) groups. Also, Kruskal-Wallis H test was used to compare nonparametric continuous variables in more than two different groups. The Spearman correlation coefficient (r) to assess the strength of the correlations between pairs of variables. Significant predictors in the univariate analysis were entered into regression model. For all above-mentioned statistical tests the results were considered significant when the probability of error is less than or equal 5 % ($p \leq 0.05$).

Results

The results showed no statistically significant difference in the clinical and demographic criteria between SLE with and without nephritis except for gender as all the cases in the NN group were females while males represented 10.5 % of the LN group (p = 0.047). The included patients had a mean age of 32.8 ± 12.6 and 30.7 ± 9.8 in the NN and LN groups, respectively. Comparing the clinical profile of patients in the LN and NN groups, there was no statistically significant difference in the incidence of diabetes mellitus, cardiac manifestations, respiratory manifestations, haematological manifestations, musculoskeletal manifestations, mucocutaneous manifestations and vascular manifestations (Table 1).

The prevalence of hypertension (HTN) was higher in LN patients (57.9 %) as compared with the NN group (21.4 %) (p = 0.002). The prevalence of neurological manifestations was 42.1 % in the LN patients and 21.4 % in the NN group (p = 0.05). In both LN and NN groups, male gender, patients with neurological, respiratory and vascular manifestations, as well as patients with urinary casts and protein of more than 500 mg/day had statistically significant higher SLEDAI-2K Score (Table 2). Urinary protein was significantly higher in LN patients with a median of 2.100 mg/day as compared to the NN patients (p ≤ 0.001). There was no statistically significant difference in the haemoglobin level, RBC count, total and differential WBC count, platelet count, serum Na level, ANA and complement (C3, C4 count between the two comparison groups (Table 3).

Table 1: Basic clinical data and extra renal manifestations of the studied groups (n = 80)

Parameters	Non-nephritis SLE	Nephritis SLE	Sig
Blood pressure			
Hypotensive	2 (4.8 %)	0 (0.0 %)	MC = 12.1
Normal range	31 (73.8 %)	16 (42.1 %)	p = 0.002*
Hypertensive	9 (21.4 %)	22 (57.9 %)	
Diabetes mellitus			
Yes	4 (9.5 %)	0 (0.0 %)	FET**
No	38 (90.5 %)	38 (100.0 %)	p = 0.12
Neurological manifestations			
Yes	9 (21.4 %)	16 (42.1 %)	χ^2 = 4.00***
No	33 (78.6 %)	22 (57.9 %)	p = 0.05
Cardiac manifestations			
Yes	7 (16.7 %)	6 (15.8 %)	χ^2 = 0.01
No	35 (83.3 %)	32 (84.2 %)	p = 0.91
Respiratory manifestations			
Yes	14 (33.3 %)	17 (44.7 %)	χ^2 = 1.10
No	28 (66.7 %)	21 (55.3 %)	p = 0.29
Haematological manifestations			
Yes	19 (45.2 %)	21 (55.3 %)	χ^2 = 0.80
No	23 (54.8 %)	17 (44.7 %)	p = 0.37
Musculoskeletal manifestations			
Yes	32 (76.2 %)	33 (86.8 %)	χ^2 = 1.50
No	10 (23.8 %)	5 (13.2 %)	p = 0.26
Mucocutaneous manifestations			
Yes	29 (69.0 %)	25 (65.8 %)	χ^2 = 0.09
No	13 (31.0 %)	13 (34.2 %)	p = 0.76
Vascular manifestations			
Yes	5 (11.9 %)	4 (10.5 %)	χ^2 = 0.04
No	37 (88.1 %)	34 (89.5 %)	p = 0.85
Lower limb oedema			
Yes	16 (38.1 %)	19 (50.0 %)	χ^2 = 1.10
No	26 (61.9 %)	19 (50.0 %)	p = 0.28

*Sig: test of significance; SLE: systemic lupus erythematosus; *MC: Monte Carlo test; ** FET: Fisher's Exact test; ***χ^2: Chi-square test;*

Table 2: Association between trimethylamine N-oxide (TMAO) and other parameters of the studied group

Parameters	TMAO	Sig
Gender		
Male	8.8 (7.3 – 32.4)	Z = 0.89
Female	8.1 (4.2 – 46.2)	p = 0.39
Blood pressure		
Hypotensive	10.7 (8.6 – 12.8)	KW = 1.6
Normal range	8.1 (5.6 – 46.2)	p = 0.44
Hypertensive	8.1 (4.2 – 32.4)	
Diabetes mellitus		
Yes	8.4 (6.3 – 39.2)	Z = 0.17
No	8.2 (4.2 – 46.2)	p = 0.89
Neurological manifestations		
Yes	8.0 (4.2 – 32.4)	Z = 1.4
No	8.3 (5.6 – 46.2)	p = 0.16
Cardiac manifestations		
Yes	8.6 (6.5 – 27.6)	Z = 0.54
No	8.1 (4.2 – 46.2)	p = 0.59
Respiratory manifestations		
Yes	8.4 (4.2 – 39.2)	Z = 0.89
No	8.0 (6.1 – 46.2)	p = 0.37
Haematological manifestations		
Yes	8.6 (6.3 – 46.2)	Z = 1.2
No	7.9 (4.2 – 39.2)	p = 0.23
Musculoskeletal manifestations		
Yes	8.3 (6.1 – 46.2)	Z = 2.1
No	7.5 (4.2 – 12.1)	p = 0.04
Mucocutaneous manifestations		
Yes	8.3 (5.6 – 46.2)	Z = 1.5
No	8.0 (4.2 – 20.4)	p = 0.14
Vascular manifestations		
Yes	7.5 (4.2 – 10.9)	Z = 1.5
No	8.2 (5.6 – 46.2)	p = 0.14
Lower limb oedema		
Yes	8.3 (4.2 – 46.2)	Z = 0.54
No	8.1 (5.6 – 27.6)	p = 0.59
Proteinuria (mg/day)		
< 500 mg/day	8.1 (5.6 – 39.2)	Z = 0.18
≥ 500 mg/day	8.3 (4.2 – 46.2)	p = 0.86
Casts in urine		
Yes	8.4 (5.6 – 46.2)	Z = 0.46
No	8.1 (4.2 – 39.2)	p = 0.64

Z: Mann Whitney test; KW: Kruskal-Wallis test; Sig: test of significance;

Patients with LN exhibited higher titres of anti-dsDNA. The median level of TMAO was 8.1 ng/mL, 8.3 ng/mL and 16.6 ng/mL in the NN, LN and control group respectively (p = 0.01) (Figure 1).

There was no significant difference in the TMAO level in male and female SLE patients. Increased TMAO levels have been only associated with musculoskeletal manifestations in SLE patients.

Table 3: Spearman's correlation between trimethylamine N-oxide (TMAO) and other parameters in LN and NN groups (n = 80)

Parameters	TMAO in LN		TMAO in NN	
	r	p	r	p
Age	-0.17	0.28	0.030	0.88
Hb (g/dL)	-0.11	0.48	0.200	0.24
RBC ($\times 10^6$/mm^3)	-0.13	0.41	-0.380	**0.02**
WBC ($\times 10^3$/mm^3)	-0.09	0.56	-0.030	0.85
Lymphocytes ($\times 10^3$/mm^3)	0.25	0.11	-0.080	0.65
Platelet count ($\times 10^3$/mm^3)	0.26	0.09	0.090	0.59
Serum creatinine level (mg/dL)	0.05	0.75	0.070	0.66
Serum Na level	0.16	0.30	-0.100	0.56
Serum K level	-0.05	0.77	0.100	0.55
ANA	0.02	0.89	-0.010	0.95
Anti-ds DNA	0.20	0.20	0.290	**0.02**
C3	0.06	0.69	0.040	0.80
C4	0.15	0.33	0.010	0.94
Urinary protein (mg/day)	-0.24	0.13	-0.007	0.97
Count of WBCs or pus cells in blood urine	-0.05	0.78	-0.060	0.74
Count of RBCs in urine	-0.26	0.09	-0.230	0.17
SLEDAI-2K score	-0.15	0.36	-0.020	0.89
Class of renal biopsy	-	-	0.120	0.48
Number of glomeruli	-	-	0.090	0.59
Activity index	-	-	-0.150	0.40
Chronicity index	-	-	-0.030	0.89

r: correlation coefficient; Group NN: included patients who were diagnosed as SLE without nephritis; Group LN: included patients who were diagnosed as lupus nephritis; Hb: haemoglobin; RBC: red blood cells; WBC: white blood cells; SLEDAI: Systemic Lupus Erythematosus Disease Activity index; ANA: antinuclear antibodies;

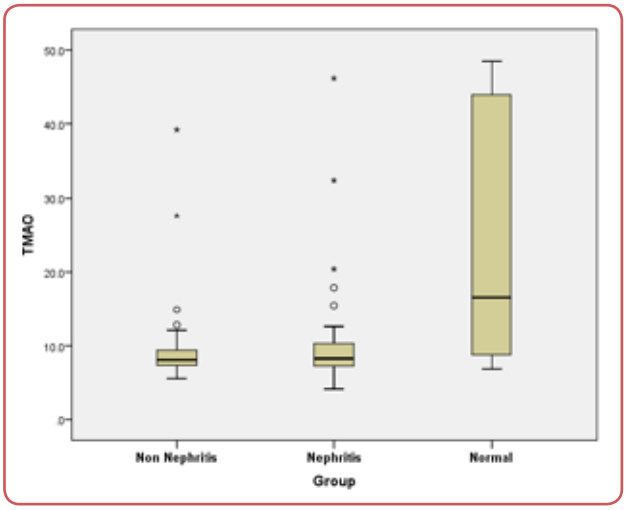

Figure 1: Serum trimethylamine N-oxide (TMAO) concentration in non-nephritis SLE, nephritis SLE and control group

TMAO positively correlated to the Anti-ds DNA titres and negatively correlated to the blood and urinary RBCs in the LN group. Nevertheless, other parameters did not reveal any significant correlation with TMAO (Table 3).

Table 4: Multiple linear regression with trimethylamine N-oxide (TMAO) as dependent variable in the studied group

Predictor (s)	β	t	p
RBC	-0.12	1.1	0.31
Count of RBCs in urine	-0.14	1.2	0.25
Anti-ds DNA	0.27	2.4	0.02*
Musculoskeletal manifestations			
Yes	-0.16	1.4	0.15
No (r)			

R^2 = 0.14; F = 3.1; Constant = 1.1; Overall p = 0.02; RBC: red blood cells;

In the Multiple linear regressions with TMAO as a dependent variable in the studied group, only anti-dsDNA revealed a statistically significant association with TMAO levels (Table 4).

Discussion

The microbiota, as an enormous human body population, plays a great role in human health and disease. This involves mastering the development of the immune system and homeostasis. Through developing a number of metabolites, microbes can support human health and prevent disease, but it can be a double-edged weapon for the host.[28] Recent advancements in metabolomics have widened understanding of the metabolites developed or metabolised by gut microbes which can function in a wide range of diseases, including autoimmune diseases, as significant immune regulators or activators.[10]

Serum TMA levels were found to be elevated in a group of patients with active rheumatoid arthritis (RA).[11] In psoriatic arthritis patients, serum TMAO correlated to disease activity in both skin and peripheral joints.[12] In humans, TMA is synthesised exclusively by gut microbiota from dietary nutrients contained in high-fat foods including choline, L-carnitine and other TMA-containing nutrients. TMAO has been recognised as a metabolite that serves as a potent RA discriminator in the urine compared to healthy subjects. Whether TMAO could help as a discriminator of SLE from healthy subjects or could discriminate LN from NN patients and whether it has an association with the disease severity including clinical, laboratory and histopathological criteria; this was the aim of this study. In the present study, TMAO levels have been evaluated in patients with SLE with and without nephritis in comparison with controls.

It has been previously found that urinary TMAO levels were higher in SLE patients than in healthy participants. This result suggests that gut microbiota and specific dietary nutrients that enhance TMAO generation are associated with disease severity.[29] This contradicts the results of the present study as it has been found that TMAO levels were significantly higher in healthy controls compared to SLE patients with and/or without nephritis. Disturbances in the composition and balance of the microflora can occur due to unhealthy lifestyle (eg unhealthy diet, low degree of physical activity and stress), consequent diseases or the use of some medications can lead to a number of disorders both locally in the gut as well as systemically.[30] This reflects an important limitation of the present study; the assessment of dietary pattern in patients and healthy controls and its effect on the gut microbiome and its derived metabolites as TMAO. As an instance, a known confounding factor is the fact that certain types of fish intrinsically contain very high amounts of TMAO[31] and thus having such a diet can affect the TMAO assessment. Another important point that should be considered the polypharmacy usually used by SLE patients and the effect of the commonly used immunosuppressive medications on the gut microbiome.

Whether certain immunosuppressive could suppress the normal gut microbiota; this raises a doubt to the largely supporting evidence to the validity of TMAO as a biomarker. Furthermore, whereas the role of bacterial-derived TMAO in cardiovascular disease pathogenesis has recently gained significant interest, it has not remained indisputable. Jia and colleagues recently used a Mendelian randomisation approach to find that genetically predicted higher TMAO was not associated with higher odds of cardiovascular disease.[32] These authors instead conclude that the observational evidence for cardiovascular diseases may be due to confounding or reverse causality.[30] The correlations between TMAO and clinical-laboratory parameters of LN and NN groups revealed only significant correlation with RBCS counts in LN patients.

Correlating the values of TMAO to the clinical-laboratory parameters of the total SLE population, a significant correlation has been found between TMAO and RBCs count, urinary RBCs and anti-dsDNA titres. The current literature suggests a regulatory effect of the gut microbiome on the haematopoietic system.[33] This might explain

the correlation between TMAO and RBCs count shown in the present study. The positive correlation between TMAO and autoantibodies such as anti-dsDNA titres has been supported by previous studies. TMAO was suggested as a diagnostic biomarker in animal models with MPO-ANCA vasculitis[34] as well as it correlated to the activity of various autoimmune disease.[35, 36] Reported that translocation of gut commensals drives IFN and anti-dsDNA in mice with lupus like disease.[36] Among the positively correlating variables to TMAO, it has been revealed in a multiple linear regression analysis with TMAO as a dependent variable that TMAO significantly predicted anti-dsDNA levels but no other parameters. Hormonal regulation of the processes of microbe control suggests that the commensal composition should vary between the two genders.[37]

In the present work, TMAO levels did not vary between male and female patients, but this can be simply attributed to the greater predominance of females in the included study sample which reflects the classic gender predominance in SLE patients. It has been previously shown that in animal SLE models, the diversity of gut microbiota differs between female and male lupus-prone mice and the female mice have more severe disease[6] and castration of male mice reversed this difference indicating a protective androgen-dependent pathway.[37]

The present study failed to report an association between TMAO and the different studied demographic and clinical parameters. An exception was the significant association between TMAO and the musculoskeletal manifestations of SLE patients. The gut derived metabolite, TMAO appears to be a major player in the various determinants of rheumatic and musculoskeletal diseases.[38] As well, raised TMAO levels have been shown to exhibit a strong negative correlation with the degree of bone mineral density (BMD) in patients with osteoporosis.[39]

In the present study, there was no significant statistical difference of TMAO levels between NN and LN patients while SLEDAI-2K score was significantly higher in LN patients. Previous studies have emphasised the prognostic value of SLEDAI-2K score particularly in LN patients.[40] TMAO has been previously identified as marker of renal medullary injury and may be an indicator of the tubulointerstitial nephritis. However, it did not show correlation to the tubulointerstitial lesions in the studied kidney biopsies. In an earlier study, TMAO has been found to be the strongest predictor of histological injury, suggesting that it may reflect the totality of renal parenchymal injury. The majority of LN patients included in this study had active proliferative lupus nephritis with class IV representing (56.8 %) and class V representing (21.6 %) of the total nephritis population. This copes with previous reports revealing a higher prevalence of proliferative class III, IV in the studied renal biopsies.[40] The exact molecular pathways linking gut microbiota to SLE and lupus nephritis are not fully elucidated. SLE patients exhibit limited gut microbiota diversity[36] however, further studies are warranted in this field.

Based on the results, the diagnostic and prognostic utility of TMAO generally in the setting of autoimmunity and specifically, in SLE faces many challenges. The reliance on TMAO as a diagnostic marker should put into consideration several dietary and comorbid conditions. This study presented two important limitations; first, the small sample size reflects the need for further studies with larger sample sizes. Second, the dietary patterns and polypharmacy might have impacted the results, however, most of the current evidence supporting the potentiality of TMAO as a diagnostic biomarker did not investigate the dietary pattern of the participants.

Conclusion

Contrary to the results of the previous studies, TMAO levels were found to be higher in healthy controls rather than SLE patients and did not discriminate between LN and NN patients as well as did not show significant correlation to the studied criteria of disease severity excluding the Anti-dsDNA titres. The possible confounding effect of the dietary pattern and ingested drugs on the gut microbiome underestimate the diagnostic and prognostic utility of TMAO as a potential marker in different diseases.

Ethics

This study was approved by the Institutional Review Board of the Mansoura Faculty of Medicine, Mansoura University (decision No MS.18.12.388, dated 6 February 2019). Written informed consent was obtained from patients prior to their participation in the study and for publishing of the anonymised patient data. The authors have followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Guidelines. The study was organised and implemented based on the adherence to the Ethical Principles for Medical Research Involving Human subjects (The Declaration of Helsinki, 8th Revision, 2013).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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