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Association of serum trimethylamine oxide with red blood cell parameters in patients with chronic kidney disease

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ABSTRACT

Objectives: The present study was designed to determine the relationship between serum TMAO levels and red blood cell (RBC) parameters in patients with chronic kidney disease (CKD).

Material and Methods: This was a case–control study involving 100 subjects comprising 50 CKD patients (25 patients with mild anemia and 25 patients with severe anemia) and 50 apparently healthy controls recruited from April to December 2023. The TMAO levels were measured using Sandwich-based Enzyme-Linked Immunosorbent Assay kit (Sulong Diagnostic Ltd, Wuham, China) while RBC parameters were measured using Automated Hematology Analyzer (Mindray BC-6800, Shenzhen, China). Data were analyzed using GraphPad Prism version 8.0 (San Diego, California, USA) and presented as mean ± SD with $P < 0.05$ considered significant.

Results: The mean value of TMAO (ng/mL) was 189.4 ± 2.4 in CKD with severe anemia, 122.3 ± 1.6 in CKD with mild anemia, and 36.4 ± 2.7 in control. Serum TMAO showed significant negative correlation with RBC ($P = 0.003$), hemoglobin (Hgb) ($P = 0.002$), hematocrit ($P = 0.004$), mean cell volume ($P = 0.0240$), mean cell Hgb ($P = 0.018$), and mean cell Hgb concentration ($P = 0.001$) but a significant positive correlation with erythropoietin ($P = 0.029$) and rticulocyte ($P = 0.022$).

Conclusion: Serum TMAO levels showed significant association with markers of anemia in CKD suggesting a potential therapeutic target for anemia in patients with CKD.

Keywords: Anemia, Inflammation, Trimethylamine oxide, Kidney, Disease, Therapy

INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem which has been ranked by the Global Burden of Disease group as the 19th leading cause of morbidity and mortality with higher prevalence and huge economic burden for families in low-and-middle-income countries such as Nigeria.[1,2] Anemia is a major contributor to high morbidity and poor quality of life associated with CKD patients and an independent risk factor for mortality[3-6] Study-based estimated prevalence of anemia is 15% in the United States CKD patients, 45–55% in the Asian CKD patients, and 50–90% in African CKD patients[7] Poor management of anemia is currently the greatest challenge faced by CKD patients in the sub-Saharan African countries such as Nigeria.[8-10]

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Anemia of CKD is characterized by an inflammation-mediated reduction in red blood cell (RBC) production, RBC survival, and/or distortion of iron metabolism[11-14] Endotoxemia is the presence of lipopolysaccharides; a major component of the outer membrane of Gram-negative bacteria released from the gut microbiota which causes systemic inflammation in the host[15] Trimethylamine oxide (TMAO) is a gut microbiota-derived endotoxin which is formed in the liver from the oxidation of trimethylamine a product of trimethyl-alkyl ammonium compounds (choline, carnitine, and betaine) by flavin-containing monoxygenases (FMOI and FMO2) which is up regulated during inflammation in many diseases including CKD[16-18] There is a paucity of data on the role of TMAO in anemia of CKD particularly for patients within the sub-Saharan African region such as Nigeria. The present study was, therefore, designed to determine the association between serum levels of TMAO and RBC parameters in CKD patients.

MATERIAL AND METHODS

Ethical considerations

Ethical approval was obtained from the Research Ethical Review Committee of the Enugu State University of Science and Technology (ESUT) Teaching Hospital, Enugu, Nigeria with registration number: (ESUT NP/C-MAC/RA/034/Vol.4/347). The nature and objectives of the study were duly explained to the subjects before recruitment into the study.

Study setting

The study was carried out in the ESUT Teaching Hospital, Enugu, Nigeria. The ESUT Teaching Hospital is the major tertiary health facility for the State and is located at the center of the capital city also known as Enugu for easy accessibility to residents. Enugu State is made up of three senatorial zones, namely, Enugu East, Enugu West, and North. The senatorial zones are divided into seventeen Local Government Areas comprising 450 communities. The State derived her name from the name of her capital and largest city Enugu. It has an area of 7,161 km^2 with a population of 3,267,837, lies between longitudes 6°30^{1}E and latitudes 5°15^{1}N and 7°15^{1}E. It is bordered by Abia state and Imo state to the South, Ebonyi to the East, Benue and Kogi states to the North and Anambra to the West. It comprises mainly the Igbo speaking tribe of South Eastern Nigeria, about 50% of which lives in the rural areas.[19]

Study design

This was a case-controlled study carried out on patients attending the Nephrology Clinic and/or admitted in the clinic between Aprils to December 2023. The study comprised 100 subjects which were divided into 50 cases which were patients with CKD (25 pre-dialysis CKD subjects with mild anemia and 25 pre-dialysis CKD patients with severe anemia) and 50 apparently healthy individuals as controls.

Sample size

A minimum sample size (definite number) that was representative of the population was determined by the relation.[20]

$$nf=\frac{n}{1+\frac{n}{N}}$$

Where
nf = Definite number of patients (minimum sample size)
N = Number of CKD patients in the registry of the Nephrology Clinic in ESUT identified to be 43 patients.
n = The sample size for a definite population

$$\frac{Z^2(P)(1-P)}{d^2}$$

Where
Z = 1.96 critical value at the level of significance (95% confidence interval)
P = 77.5% prevalence of anemia in CKD patients in Enugu[21]
d = 0.05 (tolerable error)

$$\text{Substituting } n = \frac{1.96 \times 1.96(0.775)(1-0.775)}{0.05 \times 0.05}$$

$$nf = \frac{267}{1+\frac{267}{43}} = 44.5$$

Considering a response rate of 90%, the sample size was further adjusted to accommodate attrition using the relation
Ns = nf/r

Where
Ns = Adjusted sample size for a response rate
Nf = Calculated sample size
r = The anticipated response rate of 90% (0.09).

Substituting into the relation

$$Ns = \frac{44.5}{0.9} = 50$$

Therefore, a total of 100 subjects comprising of 50 cases (25 cases with mild anemia and 25 cases with severe anemia) were recruited for the study.

Inclusion criteria

1. Newly diagnosed patients with CKD who were 18 years and above and have been diagnosed with anemia as defined by KD0Q1 criteria[22]

2. CKD patients who did not have record of hemorrhagic episodes within past 2 weeks of study commencement (minimum period for return to normal physiology of the system)
3. CKD patients who do not have malignancy or known hematological disorder
4. CKD patients and apparently healthy individuals who gave informed consent to participate.

Exclusion criteria

1. CKD patients on dialysis and/or had previous erythropoietin (EPO) or iron substitution therapy
2. CKD patients who had been transfused within the past 4 weeks (the average days of viability for transfused blood in a recipient being 21 days).
3. CKD patients on angiotensin converting enzyme inhibitors especially enalapril (a potential cause of inadequate EPO response).
4. CKD patients who did not give informed consent to participate.

Blood sample collection

10 mL of blood was drawn aseptically by venipuncture, 5 mL were dispensed into EDTA bottle for estimation of the markers of anemia, and the remaining 5 mL was centrifuged and plasma dispensed into plain bottles stored at −20°C for estimation of serum TMAO levels.

Measurement of RBC parameters

The various RBC parameters were determined by performing full blood count using the 5-Part Differential Automated Hematology Analyzer (Mindray BC-6800, Shenzhen China).

Measurement of serum TMAO and EPO levels

The serum TMAO and EPO levels were determined by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique using kit purchased from Sunlong Diagnostic Ltd., China (Catalog Number: BF8592V and LY 64190K, respectively) and absorbance at 450 nm read using the ELISA Microplate Reader (Mindray-96A,Shenzhen China).

Data analysis

Data were analyzed using GraphPad Prism Version 8 (San Diego, California, USA) and presented as mean ± SD with statistical significance set at $P < 0.05$. The Kolmogorv–Smirnov test was used to determine the normality of distribution of the data. One-way analysis of variance was used to determine the mean, standard deviation, and any significant differences in data among the study cases and controls while the Pearson's correlation test was used to determine the relationship between the various RBC parameters and serum levels of TMAO in the subjects.

RESULTS

As revealed by the results in Table 1, the mean TMAO, EPO, reticulocyte, and creatinine levels of CKD patients with severe anemia were significantly higher than those of CKD patients with mild anemia and the controls. The mean levels of the RBC, hemoglobin (Hgb), hematocrit (HCT), mean cell volume (MCV), mean cell Hgb (MCH), and MCH concentration (MCHC) in the CKD patients with severe anemia were significantly lower than those of CKD patients with mild anemia and control subject. With the exception of MCH, other RBC parameters (RBC, Hgb, HCT, MCV, and MCHC) were significantly lower in CKD patients with severe anemia than in CKD patients with mild anemia. HMGB1 showed moderate to strong negative correlations with RBC, Hgb, HCT, MCV, MCH, and MCHC but a strong positive correlation with creatinine, EPO, and reticulocyte count, as shown in Table 2.

DISCUSSION

Correlations of disease and disease severity with TMAO protein levels in humans have been advancing but there is currently a paucity of data on the correlation of serum TMAO levels with anemia of CKD particularly for the Nigerian population. We recorded a significant increase in the serum TMAO protein levels in the CKD subjects compared to the normal subjects in the present study. This

Table 1: Levels of trimethylamine oxide and red cell parameters in study groups.

Group	Control (*n*=50)	CKD with mild anemia (*n*=25)	CKD with severe anemia (*n*=25)
HCT (%)	6.4±2.0	22.8±4.2[a]	46.2±9.1[a,b]
Hgb (g/dL)	14.0±0.7	9.1±0.6[a]	6.5±0.6[a,b]
MCV (fl)	95.3±3.3	79.4±5.1[a]	76.3±3.2[a,b]
MCH (pg)	27.9±1.4	23.8±2.2[a]	23.1±2.2[a]
MCHC (g/dL)	33.2±1.2	28.9±2.1[a]	27.6±1.6[a,b]
RBC ($\times 10^{12}$/L)	5.3±0.6	3.5±0.5[a]	2.2±0.3[a,b]
Reticulocyte (%)	1.1±0.42	2.3±0.7[a]	5.9±1.4[a,b]
Creatinine (μmol/L)	86.8±9.4	312±26.2[a]	555.6±113[a,b]
EPO (mU/L)	22.6±2.5	35.5±5.3[a]	60.8±11.3
TMAO (ng/mL)	189.4±2.4	122.3±1.6[a]	36.4±2.7[a,b]

CKD: Chronic kidney disease, TMAO: Trimethylamine oxide, EPO: Erythropoietin, HCT: Hematocrit, Hgb: Hemoglobin, MCV: Mean cell hemoglobin, MCH: Mean corpuscular hemoglobin MCHC: Mean cell hemoglobin concentration, RBC: Red blood cell, [a]Severe or mild CKD group significantly different compared to control, [b]Severe CKD significantly different compared to mild CKD

Table 2: Association of TMAO level and red blood cell parameters in the CKD group (n=50).

TMAO (ng/mL) parameter	R-value	*P*-value
RBC ($\times 10^{12}$/L)	−0.875	0.003
HCT (%)	−0.877	0.002
Hgb (g/dL)	−0.695	0.004
MCV (fl)	−0.779	0.024
MCH (pg)	−0.635	0.018
MCHC (g/dL)	−0.738	0.017
Reticulocyte (%)	0.309	0.022
EPO (mU/L)	0.786	0.029
Creatinine (µmol/L)	0.909	0.001

CKD: Chronic kidney disease, R: Pearson correlation co-efficient, TMAO: Trimethylamine oxide, EPO: Erythropoietin, RBC: Red blood cell, Hgb: Hemoglobin, HCT: Hematocrit, MCV: Mean cell volume, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration, Correlation is significant at $P<0.05$

is similar to the findings of other studies which reported increased TMAO levels in CKD subjects compared to healthy control subjects.[23-25]

We also observed a significant increase in the serum TMAO levels in the CKD subjects with severe anemia compared to those with mild anemia. This finding suggests that the serum TMAO levels may reflect the degree of ineffective erythropoiesis and severity of anemia in CKD.

Furthermore, the observed significant negative correlation of serum TMAO levels with the HCT count, Hgb count, RBC count, MCV, and MCH concentration and a significant positive correlation with the reticulocyte count and EPO levels suggests that high TMAO levels may be a major contributory factor to the development of anemia in CKD while low serum TMAO levels may be a major limiting factor to the development of anemia. This suggests that modulation of serum TMAO levels in CKD may limit the development of anemia and offer a novel therapy; however, further studies with larger populations are needed to ascertain this.

Limitations

A limitation of the present study is that we have studied patients in a single center and were unable to determine the profile of micro-organisms that formed the microbiota of the study subjects as well as other factors such as type of diet which could as well affect serum TMAO levels.

CONCLUSION

Serum TMAO levels have a significant relationship with markers of anemia in CKD and may reflect the severity of anemia in patients. Serum TMAO levels may be a link between dysbiosis and anemia in CKD suggesting that modulation of gut bacterial-derived TMAO may represent a new approach in the management of anemia in CKD.

Ethical approval

The research/study approved by the Research Ethical Review Committee of the Enugu State University of Science and Technology (ESUT) Teaching Hospital, Enugu, Nigeria, number ESUT NP/C-MAC/RA/034/Vol.4/347, dated 3rd April 2023.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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