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RESEARCH ARTICLE

FULL BLOOD COUNT AND SELECTED TRACE ELEMENTS IN PATIENTS INFECTED WITH PULMONARY TUBERCULOSIS ATTENDING INFECTIOUS DISEASES HOSPITAL, IKOT EKPENE, AKWA IBOM STATE, NIGERIA

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Abstract

Background and Aims: Full blood counts and some trace elements were assessed in *Mycobacterium tuberculosis* infected patients attending Infectious Disease Hospital, Ikot Ekpene, Akwa Ibom State, Nigeria. The rationale for the study was to assess and provide information on haematological parameters, zinc, copper and selenium of tuberculosis infected patients.
Materials and Methods: Duration and use of drugs *Mycobacterium tuberculosis* therapy, age and gender were evaluated. Two hundred (200) subjects, aged 18-65 years comprising of 100 *Mycobacterium tuberculosis* infected patients on anti-tubercular, 50 drugs naive *Mycobacterium tuberculosis* infected subject and 50 apparently healthy non-infected patients who served as control subjects were recruited for this study. Informed consent was obtained from all the participants and questionnaires administered.
Results: Demographic characteristics showed more males (71%) being infected than females (29%). Married individual showed more preponderance. Those with secondary education were 42% and business men/women were 36.7%. Results showed significantly lower values ($p<0.05$) for RBC, haemoglobin concentration, hematocrit, Mean Cell volume, Mean Cell Hemoglobin and Mean Cell Haemoglobin Concentration in *Mycobacterium tuberculosis* infected patients with least values in drug naive *Mycobacterium tuberculosis* infected patients while total white blood cells and the differential white blood cells were significantly increased ($p<0.05$) in *Mycobacterium tuberculosis* infected patients when compared to the controls.
Conclusion: This study showed that tuberculosis had significant effects on haematological parameters and selected trace elements analyzed hence the need to consider supplementation to ameliorate anemia, zinc and selenium deficiencies.
Keywords: Blood counts, *Mycobacterium tuberculosis*, Nigeria, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is an old yet still widespread illness that continues to be one of the leading causes of death worldwide. It is produced by *Mycobacterium tuberculosis* and it is genetically linked to the species *M. Canettii*, *M. Africanum*, *M. microti*, *M. bovis*, *M. caprae*, and *M. pinnipedii*. *M. tuberculosis* is referred to as Koch's bacillus in honor of Robert Koch's groundbreaking research[1]. It is extremely contagious, and infection happens through inhalation and the invasion of alveolar macrophages with a low dose (1-10 bacilli)[2]. Most cases of Tuberculosis affect the lungs, leading to pulmonary Tuberculosis (TB). Nonetheless, extrapulmonary TB can also manifest in lymph nodes, pleural cavities, bones and joints, the central nervous system, intra-abdominal organs, and so on. Typical symptoms are diverse, encompassing general systemic signs (fever, weakness, malaise, weight loss, hematologic cancers, cough, and metabolic issues) as well as more specific indicators tied to the infection site(s). Tuberculosis can manifest in a range of forms, from asymptomatic infection to a severe, life-threatening condition[3,4]. From a clinical and public

health standpoint, TB patients are practically categorized into latent TB infection, an asymptomatic and non-contagious state, or active TB disease, which is contagious (specifically in active pulmonary TB)[5]. Tuberculosis is the top cause of global mortality from a single infectious agent, even exceeding HIV[6,7]. For the last 25 years, it has been regarded as a worldwide public health crisis[8,9].

Haematological measurements offer physiological details regarding the blood profile and the reticulo-endothelial system and are essential elements of an individual's health evaluation[10]. They are utilized in the detection of anemia, blood disorders, immune system diseases, and infections[11]. The utilization of reference values as proxy indicators is especially crucial for tracking disease advancement and assessing responses to treatment strategies in the management of infectious diseases. These are typically evaluated with a full blood count/complete blood count. The most frequent parameters include: total red blood cell count, total white blood cell count, haemoglobin levels, haemato-crit, mean corpuscular volume, mean corpus-cular haemoglobin, mean corpuscular haemoglobin concen-tration, total platelet count, mean platelet volume, and differential white cell count.

Trace elements are minerals found in living tissues in minimal quantities. They are technically classified as minerals needed in quantities ranging from 1 to 100 mg/day for adults or constitute less than 0 to 0.1% of total body weight[12,13]. According to WHO classification, they can be categorized as: essential elements, likely essential elements, and potentially harmful elements. These consist of chromium, copper, zinc, selenium, cobalt, molybdenum, iodine (crucial elements), manganese, silicon, nickel, boron, vanadium (likely crucial elements), fluorine, lead, cadmium, mercury, aluminum, arsenic, tin, lithium (possibly harmful elements)[14]. Essential trace elements serve various crucial functions, including acting as structural parts of vitamins (e.g., Cobalt), serving as cofactors in metallo-enzymes like glutathione peroxidase (e.g., Selenium), and functioning as catalytic components in many enzymes (e.g., Zinc and Copper)[15,16]. Trace elements are believed to affect the human body's susceptibility to the causes and outcomes of various infections due to their immunomodulatory functions[17]. Lack of various vital trace elements has been linked to reduced immunity to Tuberculosis infection.

METHODS

A hospital based comparative cross-sectional study was conducted at Infectious Disease Hospital located at Ikot Ekpene, Akwa Ibom State, Nigeria. The study population consisted of 150 pulmonary Tuberculosis patients confirmed with GeneXpert MTB/RIF assay. One hundred (100) patients were on Tuberculosis regimen while 50 patients were drug naïve. Fifty (50) apparently healthy participants that matched the sex and age of the *M. tuberculosis* patients were enrolled as controls. Blood samples were collected into EDTA containers by venepuncture after informed consent and pretest counselling for full blood counts and plain containers for zinc, copper and selenium assay. Sera from the plain containers were frozen at -20 degrees and later transported under cold chain to International Institute of Tropical Agriculture, Ibadan, Oyo State for analysis using Atomic Absorption Spectrophotometer.

Full blood count was assayed using Mindray BC-5380. It is an automated multi-parameter blood cell counter for *in vitro* diagnostic use in clinical laboratory.

Zinc, copper and selenium were assayed using atomic absorption spectro-photometric technique (Buck Scientific, 205 atomic absorption spectrophotometer, USA, 2004). GeneXpert assay procedure was used for detection of *M. tuberculosis*

Ethical clearance

Ethical approval was obtained from the Ministry of Health, Akwa Ibom State. All parti-cipants were informed about the aim of the study and informed consent was duly signed by the participants. Confidentiality was kept and participation was voluntary.

Statistical analysis

Mean, standard deviation, Student t-test and Analysis of Variance was done using SPSS for windows version 25 package (SPSS Incorporated, Chicago, USA). The Critical level of significance is $p<0.05$. Results obtained are presented in Tables.

RESULTS

This research examined some haematological parameters and selected trace elements of *M. tuberculosis* infected patients at Infectious Disease Hospital, Ikot Ekpene, Akwa Ibom State. Non- *M. tuberculosis* individuals were used as controls. Parameters assayed were Haemoglobin (Hb), Packed cell volume (PCV/haematocrit), Red cell count (RBC) Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total white blood cell count (WBC), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets, Zinc, Copper and Selenium. A total of 200 patients were enrolled for the study comprising 100 *M. tuberculosis* infected patients on drugs, 50 *M. tuberculosis* infected patients on drugs and 50 control patients. Table 1 shows the demographic characteristics of the studied patients. The maximum age of the patients with Tuberculosis was 21 years while the maximum was found to be 64 years. On the other hand, the control ranged from 27 years to 63 years. There was male preponderance in Tuberculosis infected patients (71%). Tuberculosis patients on drug who had been on treatment for 1-2 months, 3-4 months and >4 months constituted 47%, 31%, and 22% of the Tuberculosis patients on treatment. Table 2 shows haematological parameters of Tuberculosis patients and control. Analysis of variance showed significant difference ($p<0.05$) in red cell count, hemoglobin, packed cell volume (PCV/haematocrit), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell count, absolute lymphocyte, neeutrophil, monocyte, basophil and the controls. Post hoc analysis showed significant

difference in RBC, Hb, Haemacrit, MCH, TWBC and monocyte count among the three categories of the studied patients: TB patients on drugs, drug naïve TB patients and the apparently healthy controls. The MCV, Neutrophil of the TB patient on drugs is comparable with that of the control while those that are drug naïve differ from the duo. On the other hand, the MCHC of the TB patients on drug and those that are treatment naïve were comparable. Table 3 shows some trace elements among the three categories of patients studied. Analysis of variance showed significant (p<0.05) difference in the mean values of Zn, Cu and Se among the studied patients. While the post hoc analysis showed significant difference among the three groups in Zn and Se, the mean Cu values of Tb patients on drugs and those of the control were comparable and both differ from that of drug naïve TB patients. Table 4 displays haematological parameters of TB infected patients based on gender. All haematological parameters were comparable among males and females (p>0.05) except for RBC, Hb, Haematocrit and MCH. The four parameters were significantly higher in male than in female. Table 5 shows the serum level of zinc, copper and selenium of Mycobacterium infected patients based on gender. All three trace elements were comparable among males and females (p>0.05). Tables 6 haematological parameters of *M. tuberculosis* patients on drug based on treatment duration. Analysis of variance showed significant difference in RBC, Hb, hematocrit, TWBC, lymphocyte, neutrophil, mono-cytes and eosinophil. The mean Hb, hemato-criand TWBC differ among the three durations of treatment (1-2 months, 3-4 months and >4 months). On the other hand, the RBC and neutrophil counts of 3-4 months and >4 months were comparable and both differ from that of 1-2 months treatment category. Table 7 serum level of some trace elements of *M. tuberculosis* patients on drug based on treatment duration. Zinc, Copper and Selenium all showed significant difference using analysis of variance (p<0.05). The mean Cu value of the 1-2 months category was significantly higher than the 3-4 and >4 months category and the later two were comparable with each other (p>0.05).

Table 1: Demographic characteristics of pulmonary tuberculosis patients and controls.

Parameters	TB infected patients n=150	TB negative patients n=50
Age (years)	44.50±9.9	43.60±3.7
Range	21 – 64	27 – 63
Gender		
Male	107 (71.3%)	34 (68%)
Female	43 (28.7%)	16 (32%)
Marital status		
Single	45 (30%)	12 (24%)
Married	62 (41.3%)	26 (52%)
Widowed	28 (18.7%)	8 (16%)
Divorced	15 (10%)	4 (8%)
Educational status		
Non- educated	26 (17.3%)	8 (16%)
Primary	54 (36%)	15 (30%)
Secondary	63 (42%)	22 (44%)
Tertiary	7 (4.7%)	5 (10%)
Occupation		
Civil servant	14 (9.3%)	9 (18%)
Business man/woman	55 (36.7%)	21 (42%)
Students	19 (12.7%)	6 (12%)
Artisans	35 (23.3%)	11 (22%)
Unemployed	27 (18%)	3 (6%)

Table 2: Haematological parameters of pulmonary tuberculosis patients and controls.

Parameters	TB patients on drugs n=100	TB drug naive patients n=50	Control patient n=50	*p*-value
RBC ($x10^{12}$ /L)(3.8-6.5)	$4.4±0.5^a$	$3.9±0.8^b$	$4.7±0.3^c$	0.00*
Hb (g/dl)(12-16)	$12.1±1.4^a$	$9.8±2.4^b$	$14.3±0.8^c$	0.00*
Haematocit (l/l)(0.36-0.54)	$0.39±4.3^a$	$0.32±7.5^b$	$0.43±2.9^c$	0.00*
MCV (FL)(80-100)	$88.8±6.8^a$	$82.9±13.9^b$	$92.1±4.5^a$	0.00*
MCH (Pg)(27-32)	$27.3±2.4^a$	$25.3±3.2^b$	$90.2±1.6^c$	0.00*
MCHC (g/dl)(32-36)	$30.7±1.1^a$	$30.2±1.9^a$	$32.8±1.1^b$	0.00*
TWBC ($x10^9$ /L)(4-10)	$6.4 ±1.1^a$	$9.3±2.3^b$	$5.5±0.7^c$	0.00*
Neutrophil ($x10^9$ /L)(2-7)	$3.4±1.5^a$	$6.7±2.1^b$	$3.1±0.4^b$	0.00*
Lymphocyte($x10^9$ /L)(1.5-4.5)	$2.5±0.8^a$	$1.8±0.6^b$	$2.0±0.8^a$	0.00*
Monocytes ($x10^9$ /L)(0.2-0.8)	$0.3±0.2^a$	$0.4±0.3^b$	$0.2±0.1^c$	0.00*
Eosinophil ($x10^9$ /L)(0.0-0.4)	0.2±0.1	0.2±0.2	0.1±0.1	0.073
Basophil ($x10^9$ /L)(0.0-0.1)	$0.01±0.01^a$	$0.03±0.07^b$	$0.01±0.04^{a,b}$	0.043*
Platelet ($x10^9$ /L)(150-400)	224.0±68.0	222.6±111.4	230.4±41.2	0.07

Table 3: Serum levels of zinc, copper, and selenium in pulmonary tuberculosis patients and controls.

Parameters	TB patients on drugs n=100	TB drug naive patients n=50	Control patient n=50	*p*-value
Zinc(µg/dL)(60-120)	107.6±13.6[a]	99.7±12.7[b]	114.7±6.4[c]	0.01*
Copper(µg/dL)(60-140)	122.5±9.4[a]	127.1±12.2[b]	119.0±9.8[c]	0.01*
Selenium(µg/L)(70-120)	97.9±9.4[a]	90.7±13.9 [b]	107.6±6.3[c]	0.01*

Table 4: Haematological parameters of pulmonary tuberculosis patients stratified by gender.

Parameters	Gender		t-calculated	*p*-value
	Male n=107	Female n=43		
RBC (x10^{12}/L)(3.8-6.5)	4.5±0.5	4.3±0.4	2.16	0.03*
Hb (g/dl)(12-16)	11.69±1.4	10.3±1.2	3.41	0.00*
Haematocrit (l/l)(0.36-0.54)	0.38±4.2	0.34±3.7	3.71	0.00*
MCV (FL)(80-100)	89.9±5.6	85.9±8.7	1.03	0.31
MCH (Pg)(27-32)	27.7±2.1	25.4±2.8	0.54	0.01*
MCHC (g/dl)(32-36)	30.8±1.1	30.6±1.2	0.76	0.45
TWBC (x 10^9/L)(4-10)	6.5±1.1	6.1±1.0	0.29	0.77
Neutrophil (x10^9/L)(2-7)	4.54±0.8	4.31±0.6	0.52	0.60
Lymphocyte (x10^9/L)(1.5-4.5)	1.71±1.5	1.92±0.6	0.70	0.49
Monocyte (x10^9/L)(0.2-0.8)	0.34±0.2	0.36±0.2	0.39	0.07
Eosinophil ((x10^9/L)(0.0-0.4)	0.20±0.1	0.16±0.10	1.77	0.08
Basophil (x 10^9/L)(0.0-0.1)	0.01±0.00	0.01±0.00	0.04	0.97
Platelet (x 10^9/L)(150-400)	252.0±68.1	273.4±66.4	0.81	0.65

The Zn and Se of the 1-2 months duration were significantly lower than the > 4 months category. However, the 3-4 months category were comparable with both 1-2 months and>4 months duration category.

DISCUSSION

Tuberculosis continues to be a significant public health issue in developing nations like Nigeria, as it ranks among the top causes of death from a single infectious disease[17]. This study assessed haematological parameters and certain trace elements in patients with *M. tuberculosis* and a seemingly healthy control group. There was a dominance of male individuals among the *M. tuberculosis* patients studied. This increased male to female ratio aligns with previous findings in Enugu State, Nigeria[19], and the World Health Organization Report[20], which indicate that men worldwide face a much higher risk of both contracting and succumbing to *M. tuberculosis* compared to women. In 2017, nearly 6 million adult men were infected with *M. tuberculosis*, with about 840,000 succumbing to it, while approximately 3.2 million adult women became ill and nearly 500,000 died from the illness[21]. Nonetheless, on the other hand, elevated results in females have been documented in Pakistan[18]. This gender gap can be linked to women's limited use of health care services. Women have been recorded to experience reduced access to health care services, limited educational opportunities, and lower income[22]. In this study, the most common age for *M. tuberculosis* cases was under 46 years, making up roughly 62% of all TB patients. This discovery of increased prevalence in adults of working age aligns with earlier reports from Nigeria[19] and Pakistan[18,23]. The likelihood of contracting *M. tuberculosis* rises with age, likely resulting from a greater number and frequency of contacts. In this research, there was a notable decrease in red blood cells, PCV, and hemoglobin, alongside a prevalence of anemia in most tuberculosis patients. This decrease can be linked to cytokines (like IL8, TNFα) released by macrophages targeting Tubercle bacilli, which causes reduced erythropoietic production and hinders the reticuloendothelial transfer of iron in the forming red blood cells. Additionally, part of the reduction (concerning those receiving treatment) may be linked to the effects of anti-Tuberculosis medication throughout the treatment period.

As treatment advances, there is a steady enhancement in total red cell count, Hb, and PCV approaching control levels, as shown by the results (higher values are observed in patients receiving medication compared to drug-naive TB patients). This change in Hb and PCV levels can serve as indicators to gauge treatment response[18,24,25]. This was additionally supported by markedly elevated red cell counts, Hb, and PCV as treatment duration increases. Earlier studies have reported anaemia prevalence rates of 31.9%[26] in Korea and 77%[27] in Uganda.

Table 5: Serum levels of zinc, copper, and selenium in pulmonary tuberculosis patients stratified by gender.

Parameters	TB infected patients Male n=107	TB infected patient Female n=43	t-calculated	*p*-value
Zn(µg/dl)(60-120)	105.00±1.42	105.02±1.83	0.00	0.99
Cu(µg/dl)(60-140)	123.26±1.05	122.95±1.39	0.41	0.68
Se (µg/l)(70-120)	95.37±0.94	94.5±0.88	0.60	0.53

Table 6: Haematological parameters of pulmonary tuberculosis patients on treatment according to duration of therapy.

Parameters	Duration of treatment (months)			*p*- value
	1-2; n=47 Isoniazid and Rifampicin	3-4; n=31 Ethambutol and Pyrazinamide	>4; n=22 Ethambutol and Pyrazinamide	
RBC ($x10^{12}$/L)	4.2±0.4[a]	4.5±0.4[b]	4.7 ± 0.4[b]	0.00*
Hb (g/dl)	11.3±1.2[a]	12.3±1.1[b]	13.3 ± 1.2[c]	0.00*
Haematocrit (l/l)	0.36±3.7[a]	0.40±3.4[b]	43.1±2.9[c]	0.00*
MCV (FL)	87.4±7.4	90.1±6.4	90.0±5.4	0.142
MCH (Pg)	26.9 ± 2.6	27.5±2.2	27.8±2.0	0.318
MCHC (g/dl)	30.8±1.3	30.5±1.0	30.9±1.1	0.506
TWBC (x 10^9/L)	7.1±1.0[a]	6.1±0.9[b]	5.3±0.5[c]	0.00*
Lymphocyte ($x10^9$/L)	2.4±0.8	2.8±0.7	2.3±0.5	0.046
Neutrophil ($x10^9$/L)	4.2±1.6[a]	2.7±1.0[b]	2.5±0.6[b]	0.00*
Monocyte ($x10^9$/L)	0.3±0.1[a]	0.4±0.2[b]	0.3±0.1[a,b]	0.007*
Eosinophil ($x10^9$/L)	0.17±0.15[a]	0.18±0.05[a,b]	0.21±0.17[b]	0.040*
Basophil (x 10^9/L)	0.005±0.011	0.007±0.017	0.006±0.013	0.808
Platelet (x 10^9/L)	263.5±76.0	247.9±65.0	260.3±53.9	0.605

Table 7: Serum levels of zinc, copper, and selenium in pulmonary tuberculosis patients on treatment according to duration of therapy.

Parameters	1-2; n=47 Isoniazid and Rifampicin	2-4; n=31 Ethambutol and Pyrazinamide	>4; n=22 Ethambutol and Pyrazinamide	*p*-value
Zn(µg/dL)(60-120)	103.1±6.8[a]	110.3±9.0[a,b]	113.7±5.8[b]	0.003*
Cu(µg/dL)(60-140)	132.2±7.1[a]	128.4±10.2[b]	122.1±6.8[b]	0.000*
Se(µg/L)(70-120)	95.3±10.7[a]	98.6±8.0 [a,b]	102.3±6.1[b]	0.013*

In this study, there was a notably greater total white cell count in *M. tuberculosis* patients compared to the control group, with peak values observed in the drug-naive *M. tuberculosis* patients. This is supported by the observation of leucocytosis in 42% of drug-naive patients with *M. tuberculosis*. As anticipated, the count of white blood cells rose during infection because of elevated polymorphonuclear leucocytes and macrophages, which are components of the body's immune defense strategies to fight against the encroaching bacterial population[24]. This result aligns with earlier findings[28]. Lymphopenia was seen in most drug-naive TB patients, whereas lymphocytosis was noted in most TB patients undergoing treatment. These trends suggest overall clinical enhancement due to treatment and are traits of effective therapy. This discovery aligns with earlier research[28,29]. Neutrophilia was also noted in most drug-naive *M. tuberculosis* patients and a few *M. tuberculosis* patients undergoing treatment. Neutrophilia alongside elevated total white blood cell count in mycobacterial infection indicates ongoing inflammation or inability to eliminate the bacteria[30]. Persistent inflammation in chronic *M. tuberculosis* infection has been characterized as a dysfunction of the *M. tuberculosis* specific immune response and an indicator of active disease[31]. The relationship among neutrophilia, lymphopenia, and the severity of *M. tuberculosis* disease, as indicated by chest X-ray, has been previously established[32,33].

The mean platelet count of the drug-naive TB patients was similar to that of the control group (unlike the significantly higher counts in those on medication), but thrombocytopenia occurred in 34% of the drug-naive TB patients. This finding aligns with previous reports. Different factors, including drug-induced mechanisms, bone marrow fibrosis, and hypersplenism, have been suggested as potential causes of thrombocytopenia[34]. Zinc levels were notably lower in both groups of *M. tuberculosis* patients compared to the control patients. This result aligns with earlier research[35,36]. This difference is likely caused by the redistribution of zinc from the bloodstream to other tissues, decreased hepatic synthesis of α2 macroglobulin (a zinc transport protein), or heightened production of metallothioneins, which are proteins that assist in the transfer of zinc to the liver[35,37]. This trend is further confirmed by the increased zinc concentration in *M. tuberculosis* patients receiving treatment compared to those who are drug naive. Zinc regulates metallothionein, which plays a role in the elimination of free radicals and in inflammatory processes[35,38]. A lack of zinc can increase susceptibility to *M. tuberculosis* infection and make individuals more prone to oxidative stress[35].

This research showed a markedly higher average copper level in the drug-naive TB patients compared to the control group and TB patients receiving treatment. This result aligns with earlier reports[35,39]. Nonetheless, copper has been reported to be essential for the survival of mycobacterium and is additionally utilized in reaction to infection[40]. It is an important component of the immune system that counteracts reactive oxygen species produced in reaction to *M. tuberculosis* infection[36]. Copper is an essential component that boosts immunity against macrophages. In this research, a notably lower selenium concentration was detected in both TB categories in comparison to the control. This result aligns with earlier reports[41]. Selenium plays a crucial role in the upkeep of immune functions and, as

a result, may be essential in protecting against mycobacteria.

CONCLUSIONS

This research showed modified haematological parameters and certain trace elements in patients with *M. tuberculosis.* A decrease in red blood cell count, haemoglobin, hematocrit, mean cell haemoglobin, and mean cell haemoglobin concentration, leading to anaemia, was noted in patients infected with *M. tuberculosis*. Neutrophilia and lymphopenia were observed in drug-naive patients with *M. tuberculosis.* Zinc and selenium levels decreased, while copper levels increased in patients infected with *M. tuberculosis* compared to control patients.

AUTHOR'S CONTRIBUTIONS

Asemota E: formal analysis, conceptualization, data organization. **Bassey IM:** conceptualization, data organization. **Ogar C:** formal analysis, data processing. **Asemota OA:** critical review. **Abunimye DA:** editing. **Obeagu EI:** conceptualization. Final manuscript was checked and approved by all authors.

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DATA AVAILABILITY

The empirical data used to support the study's conclusions are available upon request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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