

RESEARCH ARTICLE

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Zinc–Copper ratio and Lipid peroxidation as indicators of glycaemic control in Type 2 diabetes mellitus

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Abstract

Objectives: Oxidative stress and trace element imbalance contribute to the pathogenesis of type 2 diabetes mellitus (T2DM). Zinc functions as an antioxidant and insulin cofactor, whereas dysregulated copper may amplify oxidative damage. This study evaluated serum zinc, copper, their ratio (Zn/Cu), and lipid peroxidation markers across glycaemic control categories.

Methods: In this cross-sectional study, 110 adults were classified as normoglycaemic controls (n = 40), well-controlled T2DM (HbA1c ≤ 7.0%, n = 35), and poorly controlled T2DM (HbA1c > 7.0%, n = 35). Serum zinc and copper were measured by flame atomic absorption spectrophotometry; malondialdehyde (MDA) by TBARS assay; and 8-iso-prostaglandin F₂α (8-iso-PGF₂α) by ELISA. Multivariate analysis of covariance adjusted for demographic and clinical covariates, and discriminant analysis identified variables distinguishing glycaemic groups.

Results: Poorly controlled T2DM showed higher copper (21.6 ± 0.54 μmol/L), 8-iso-PGF₂α (205.49 ± 7.23 pg/mL), and MDA (2.30 ± 0.12 μmol/L), with lower zinc (9.17 ± 0.40 μmol/L) and Zn/Cu ratio (0.43 ± 0.30) compared to other groups (p < 0.001). Zn/Cu ratio and copper showed the largest effect sizes (partial η² = 0.61 and 0.55, respectively). Zn/Cu ratio correlated negatively with 8-iso-PGF₂α (r = -0.877, p < 0.001) and MDA (r = -0.780, p < 0.001). Discriminant analysis achieved 88.7% accuracy, with Zn/Cu ratio and 8-iso-PGF₂α as key discriminators.

Conclusion: Glycaemic dysregulation in T2DM is linked to increased oxidative stress and zinc–copper imbalance. The Zn/Cu ratio effectively distinguishes glycaemic groups and serves as a cost-effective, adjunctive biomarker to complement HbA1c in risk stratification. These findings are relevant for monitoring metabolic control in resource-constrained settings, pending further validation.

Keywords: Type 2 Diabetes Mellitus; Oxidative Stress; Zn/Cu Ratio; Lipid Peroxidation; 8-iso-Prostaglandin F₂α; Malondialdehyde

Plain English summary

People with type 2 diabetes often have problems caused by “oxidative stress,” which is a kind of damage in the body linked to blood sugar control. Zinc is a mineral that helps protect against this damage and supports insulin, while copper, when out of balance, can make the damage worse.

In this study, we looked at 110 adults: some without diabetes, some with well-controlled diabetes, and others with poorly controlled diabetes. We tested their blood for zinc, copper, and signs of oxidative stress.

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We found that people with poorly controlled diabetes had higher copper and more oxidative stress, but lower zinc and a poorer balance between the two minerals. This zinc-to-copper balance, together with oxidative stress levels, proved to be very good at showing who had poor blood sugar control; almost nine out of ten people were correctly identified.

These results suggest that checking zinc, copper, and their balance could help doctors monitor diabetes more closely and spot those at greater risk of complications.

Background

Type 2 diabetes mellitus (T2DM) is a widespread metabolic disorder characterised by persistent high blood glucose levels, along with increased oxidative stress, inflammation, and blood vessel dysfunction (1, 2). In sub-Saharan Africa, the prevalence of T2DM is rising rapidly, with Nigeria currently home to over 8 million adults living with the condition; more than half of these individuals present with poorly controlled blood glucose levels, markedly increasing their risk of complications and mortality (3).

While controlling blood glucose remains the main treatment goal, research shows that micronutrient imbalances, particularly involving trace elements like zinc (Zn) and copper (Cu), can influence oxidative stress and disease progression (4, 5).

Zinc plays a key role in insulin synthesis, storage, and secretion, and it supports antioxidant enzymes like superoxide dismutase (SOD) and metallothioneins (6, 7). Zinc deficiency has been shown to impair glucose uptake, reduce insulin sensitivity, and increase oxidative stress through diminished antioxidant defence (8, 9). Conversely, copper is required for mitochondrial energy production and enzymatic functions, but excess free copper may participate in Fenton chemistry, generating reactive oxygen species that damage cellular membranes, impair insulin signalling, and exacerbate lipid peroxidation (10, 11).

The balance between these two trace elements, commonly expressed as the serum Zn/Cu ratio, has been proposed as a potential integrated biomarker of redox and metabolic homeostasis. Disruption of this ratio has been linked to increased cardiovascular risk and poor glycaemic outcomes (12).

Furthermore, lipid peroxidation markers such as malondialdehyde (MDA) and 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) provide sensitive indices of oxidative damage and are elevated in individuals with T2DM, particularly those with complications (13). However, most prior studies compare diabetic and non-diabetic populations without considering glycaemic control status as a clinically important stratifier of metabolic risk (14, 15).

Trace element status and oxidative stress responses are also influenced by genetics, diet, and environmental exposures (16, 17, 18). In sub-

Saharan Africa, where dietary mineral deficiencies are common, these factors may exacerbate redox imbalance in T2DM. Despite this, few studies in the region have investigated the relationship between zinc–copper imbalance, oxidative stress, and glycaemic control.

Importantly, previous statistical approaches have been limited by multicollinearity among biochemical markers, which complicates interpretation and weakens regression-based models. To address this, the present study incorporates multivariate techniques, particularly discriminant function analysis (DFA), to improve the classification of glycaemic phenotypes based on redox-related biomarkers. By identifying variables with the greatest discriminative power, DFA offers a more nuanced and clinically actionable understanding of trace element–oxidative stress interactions in T2DM.

This study, therefore, aims to assess serum zinc, copper, the Zn/Cu ratio, and lipid peroxidation markers (MDA and 8-iso-PGF $_{2\alpha}$) in healthy individuals, well-controlled T2DM patients (HbA1c $\leq$ 7.0%), and poorly controlled T2DM patients (HbA1c $>$ 7.0%). We hypothesise that poor glycaemic control is associated with disrupted Zn–Cu homeostasis and increased oxidative stress, highlighting potential biomarkers for disease monitoring and metabolic risk stratification.

Materials and Methods

Study Design

This was a cross-sectional observational study conducted at the Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria, between January and June 2025. A total of 110 adults aged 30–60 years were enrolled and stratified into three groups based on glycaemic status:

Healthy Controls (n = 40): Normoglycaemic individuals with no history of diabetes, fasting plasma glucose (FPG) $<$ 100 mg/dL, and glycated haemoglobin (HbA1c) $<$ 5.7%, as per American Diabetes Association (ADA) criteria (19).

Well-Controlled T2DM (n = 35): Diagnosed cases of type 2 diabetes mellitus for at least five years, receiving treatment, with HbA1c $\leq$ 7.0%.

Poorly Controlled T2DM (n = 35): Diagnosed cases of type 2 diabetes mellitus for at least five years with HbA1c > 7.0%.

The criterion of a minimum five-year diabetes duration was applied to ensure sufficient disease chronicity for evaluating trace element imbalance and oxidative stress, while minimising variability associated with early disease stages.

Participant Recruitment and Sampling

Participants with T2DM were recruited consecutively from the endocrinology outpatient clinic using hospital records and clinical consultation lists, in collaboration with the attending endocrinologist. A structured questionnaire was administered to confirm eligibility and obtain clinical and demographic data. Healthy controls were selected from hospital staff and patient relatives. Although a simple random sampling approach was applied after categorisation within each group, the selection of healthy controls represents a form of convenience sampling and may limit generalizability.

Exclusion Criteria

Participants were included if they had type 1 diabetes, gestational diabetes, chronic kidney or liver disease, recent cardiovascular events (within the last 6 months), acute or chronic inflammatory disorders, malignancies, recent use of zinc or copper supplements (within the last 3 months), cigarette smoking, or alcohol dependence.

Minimisation of Confounders

To reduce the influence of short-term dietary variation on zinc and copper levels, all participants provided fasting blood samples, and the questionnaire captured recent dietary habits to identify and exclude individuals with unusually high intake of zinc- or copper-rich foods.

Potential confounders, including dietary mineral intake and renal clearance, were addressed by excluding supplement users and those with chronic kidney disease. However, subtle variations in renal trace element handling (e.g., due to hyperglycaemia-related hyperfiltration or early tubular dysfunction in T2DM) could not be fully excluded.

Sample Size

The sample size was calculated based on expected moderate effect sizes from prior studies examining trace element differences across glycaemic groups (20). A minimum of 35 participants per group was targeted to ensure sufficient power for multivariate analysis.

Anthropometric Assessment

Participants were assessed in a standing position, barefoot, and in light clothing. Height was measured to the nearest centimetre using a non-elastic measuring tape. Weight was recorded with a digital scale (OMRON HBF-514C, Kyoto, Japan), and BMI was calculated as weight (kg) divided by height squared (m²). Waist circumference was measured in centimetres at the midpoint between the lower rib margin and iliac crest using a flexible tape during normal expiration. Blood pressure was measured using an automated sphygmomanometer (OMRON HEM-907XL, Kyoto, Japan) after five minutes of rest.

Sample Collection and Biochemical Analysis

All participants were instructed to observe an overnight fast of 8–12 hours, venous blood samples were collected between 08:00 and 10:00 hours to minimise diurnal variation and standardise conditions. Fasting status was confirmed verbally at the point of sample collection, and participants who reported non-adherence to the fasting protocol were rescheduled or excluded. A total of 10 mL of venous blood was drawn from each participant into potassium-EDTA, sodium fluoride, and plain vacutainer tubes. Samples were immediately placed in a cold chain and transported for processing under controlled laboratory conditions. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at –80°C until biochemical analysis.

Glycaemic Parameters

Fasting plasma glucose was measured using the glucose oxidase method (21), valued for its specificity, affordability, and minimal interference, making it suitable for routine use in low-resource settings. HbA1c was determined with the Diazyme enzymatic assay (22), an NGSP-certified, IFCC-aligned method offering improved specificity and reduced interference from haemoglobin variants, relevant in populations with prevalent haemoglobinopathies.

Lipid Peroxidation Markers

Serum MDA levels were quantified by the thiobarbituric acid reactive substances (TBARS) assay, a cost-effective approach for estimating lipid peroxidation. 8-iso-PGF₂α was measured using a sandwich Enzyme-Link-Immunosorbent Assay (ELISA) (23) (Elabscience, Wuhan, China), selected for its sensitivity and suitability in resource-limited laboratories.

Trace Elements

Serum zinc and copper were analysed by flame atomic absorption spectrophotometry (PerkinElmer AAnalyst 400 specifications, USA) (24) at 213.9 nm and 324.8 nm, respectively. Flame Atomic Absorption Spectrophotometry was chosen for its reliability, affordability, and applicability in low and middle-income countries' laboratory settings. Certified reference standards were used for calibration, and all glassware and instruments were acid-washed to minimise contamination. The Zn/Cu ratio was computed as the quotient of serum zinc and copper

Quality Control

All biochemical assays were conducted in duplicate. Internal quality control materials were included in each analytical run. Intra- and inter-assay coefficients of variation (CV) were maintained below 10%.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), and categorical variables as frequencies and percentages. Group comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. Chi-square tests assessed differences in categorical variables. Statistical significance was set at $p < 0.05$, with adjustments applied for multiple comparisons as appropriate.

Multivariate analysis of covariance (MANCOVA) was conducted to examine group differences in trace element levels and oxidative stress biomarkers, adjusting for covariates including age, sex, BMI, blood pressure, diabetes duration, and waist circumference. Results were presented as adjusted means $\pm$ standard error (SE), and effect sizes were reported using partial eta squared (η^2). Pearson's correlation analysis was used to explore linear associations among variables. To assess

multicollinearity among oxidative stress and trace element biomarkers, Principal Component Analysis (PCA) was performed. Components with eigenvalues greater than 1 were retained, and scree plot inspection guided factor extraction. Given the presence of high intercorrelations among the biomarkers, traditional regression analysis was not suitable.

Therefore, Discriminant Function Analysis (DFA) was employed to evaluate the discriminative power of the biomarkers in classifying individuals into glycaemic phenotypes. This approach allowed the identification of combinations of variables that best separated the groups. Standardised canonical coefficients and structure matrix loadings were examined to determine variable contributions, and model performance was assessed using classification accuracy and eigenvalue statistics.

Results

Clinical and metabolic profiles across glycaemic phenotypes

Key clinical parameters varied significantly among the three glycaemic groups (Table 1). Participants with well-controlled type 2 diabetes were significantly older than both the poorly controlled and non-diabetic control groups ($p = 0.020$), while sex distribution remained similar across groups ($p = 0.629$).

Poor glycaemic control was associated with significantly elevated systolic and diastolic blood pressures, as well as increased waist circumference (all $p < 0.001$). However, BMI did not differ significantly across groups ($p = 0.708$). As anticipated, both HbA1c and fasting plasma glucose were highest in the poorly controlled group ($p < 0.001$), confirming the validity of the glycaemic classification. The duration of diabetes did not differ between the diabetic subgroups ($p = 0.262$), suggesting that the observed metabolic disparities were not attributable to disease duration alone.

Table 1: Sociodemographic and clinical characteristics of participants across glycaemic phenotype

Variables	Poorly Controlled DM (HbA1c > 7.0%) (N = 35)	Well-Controlled DM (HbA1c < 7.0%) (N = 35)	Healthy controls (HbA1c < 5.7%) (N = 40)	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Age (years)	42.11 ^{ab} $\pm$ 5.20	46.09 ^b $\pm$ 7.66	40.78 ^a $\pm$ 10.69	0.020*
Sex (M) %	14 (40)	18 (51)	18 (45)	0.629
(F) %	21 (60)	17 (49)	22 (55)	
Duration of diabetes mellitus (Years)	6.69 $\pm$ 1.32	6.29 $\pm$ 1.38	-	0.262
Systolic blood pressure (mm/Hg)	134.40 ^a $\pm$ 14.94	127.86 ^b $\pm$ 9.51	118.13 ^c $\pm$ 7.36	< 0.001*

Diastolic blood pressure (mm/Hg)	86.83 ^a ± 12.99	80.20b ± 9.86	76.65 ^b ± 7.43	< 0.001*
Body mass index (kg/m ²)	26.78 ± 2.52	27.29 ± 2.35	26.98 ± 2.52	0.708
Waist circumference (cm)	98.37 ^a ± 5.68	95.57a ± 4.35	91.15 ^b ± 4.26	<0.001*
HbA1c	9.5 ^a ± 1.14	6.5b ± 0.34	5.1 ^c ± 0.30	< 0.001*
Fasting plasma glucose (mmol/L)	8.76 ^a ± 1.14	6.59b ± 0.63	4.73c ± 0.61	< 0.001*

Data are shown as mean ± SD or number (percentage). Group differences were tested with one-way ANOVA (Tukey post hoc) or Chi-square. Superscript letters (a, b, c) mark significant group differences ($p < 0.05$); identical letters indicate no difference.

Trace element and oxidative stress profiles across glycaemic phenotypes

Significant differences were observed across all oxidative stress and trace element parameters, even after adjusting for age, sex, BMI, blood pressure, waist circumference, and diabetes duration ($p < 0.001$ for all comparisons; Table 2).

Participants with poorly controlled diabetes exhibited markedly higher serum levels of 8-iso-PGF₂α and MDA, indicative of exacerbated oxidative stress. These markers demonstrated strong effect sizes (partial $\eta^2 = 0.535$ and 0.394 , respectively).

Serum copper levels were significantly elevated in the poorly controlled group, whereas serum zinc concentrations and the Zn/Cu ratio were notably reduced. The Zn/Cu ratio and serum copper exhibited the largest effect sizes (partial $\eta^2 = 0.616$ and 0.557 , respectively), highlighting their potential as discriminative biomarkers of metabolic dysfunction in type 2 diabetes.

Table 2: Adjusted mean levels of trace elements and lipid peroxidation markers across glycaemic phenotypes

Variables	Poorly Controlled	Well-Controlled	Healthy controls	F (df1, df2)	p-value	Partial η^2
	Diabetes mellitus (HbA1c > 7.0%) (N = 35)	Diabetes mellitus (HbA1c < 7.0%) (N = 35)	Healthy controls (HbA1c < 5.7%) (N = 40)			
	Mean ± SE (Adjusted)	Mean ± SE (Adjusted)	Mean ± SE (Adjusted)			
8-iso-PGF ₂ α (pg/mL)	205.49 ^a ± 7.23	143.02 ^b ± 7.25	96.83 ^c ± 10.81	57.45 (2,100)	< 0.001*	0.535
MDA (μmol/L)	2.30 ^a ± 0.12	1.53b ± 0.1	0.91 ^b ± 0.182	32.49 (2,100)	< 0.001*	0.394
Copper (μmol/L)	21.6 ^a ± 0.54	16.64 ^b ± 0.54	13.63 ^b ± 0.80	62.99 (2,100)	< 0.001*	0.557
Zinc (μmol/L)	9.17 ^a ± 0.40	12.21 ^b ± 0.40	14.18 ^b ± 0.60	43.55 (2,100)	< 0.001*	0.466
Zinc/Copper ratio	0.43 ^a ± 0.30	0.75 ^b ± 0.30	1.03 ^c ± 0.05	80.34 (2,100)	< 0.001*	0.616

Reference intervals are literature-derived (not population-specific) and were adapted from standard clinical references and biomarker studies: (8-iso-PGF₂α; < 150 pg/mL, MDA; 0.67–1.39 μmol/L, Copper; 11–22 μmol/L, Zinc; 11–18 μmol/L, Zinc/Copper ratio; > 1.0) (25, 26, 27).

Values are adjusted mean ± SE from MANCOVA controlling for age, sex, BMI, blood pressure, waist

circumference, and diabetes duration. Group differences were tested with Bonferroni post hoc analysis. Superscript letters (a, b, c) indicate significant pairwise differences ($p < 0.05$); identical letters indicate no difference. F-statistics and partial η^2 represent effect size and variance explained.

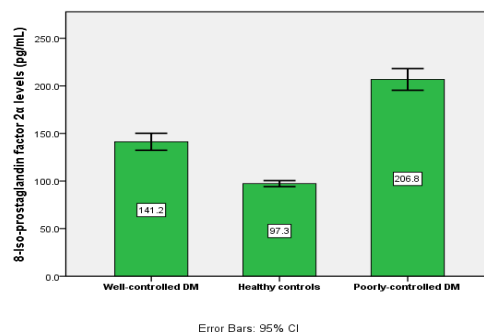


Figure 1: 8-Iso-prostaglandin factor 2α levels across glycaemic phenotype

The highest levels of 8-Iso-prostaglandin factor 2 α were observed in individuals with poorly controlled diabetes mellitus, indicating heightened oxidative stress with worsening glycaemic control.

Differences across groups were statistically significant ($p < 0.001$, Partial $\eta^2 = 0.535$). Pairwise comparisons (a, b, c) are significant at $p < 0.05$.

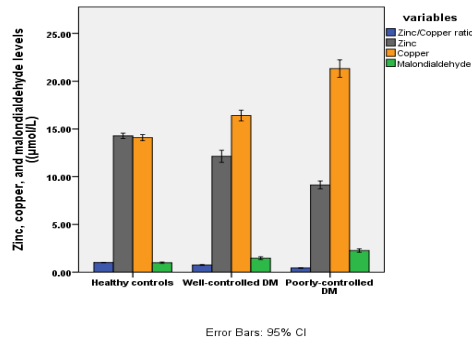


Figure 2: Serum zinc, copper, zinc/copper ratio, and malondialdehyde levels across glycaemic phenotype

Poor glycaemic control was associated with significantly elevated copper and MDA, alongside reduced zinc and Zn/Cu ratios ($p < 0.001$ for all). Effect sizes (Partial η^2): Cu (0.557), Zn (0.466), Zn/Cu (0.616), and MDA (0.394). Superscripts (a, b, c) indicate significant pairwise differences ($p < 0.05$).

oxidative stress and trace element markers (Table 3). Serum 8-iso-PGF₂ α and MDA correlated positively with copper ($r = 0.861$ and 0.776 , respectively; $p < 0.001$) and inversely with zinc and the Zn/Cu ratio. Copper and zinc were inversely correlated ($r = -0.767$), whereas the Zn/Cu ratio was negatively associated with all other markers, underscoring multicollinearity.

Correlation and multicollinearity assessment

Pearson correlation analysis revealed strong and physiologically consistent relationships among

Table 3: Pearson correlation matrix of trace elements and oxidative stress biomarkers

Variables	r	p-value
8-iso-PGF ₂ α (pg/mL) - MDA (μ mol/L)	0.815	< 0.001**
8-iso-PGF ₂ α (pg/mL) - Copper (μ mol/L)	0.861	< 0.001**
8-iso-PGF ₂ α (pg/mL) - Zinc (μ mol/L)	-0.802	< 0.001**
8-iso-PGF ₂ α (pg/mL) - Zinc/Copper ratio	-0.877	< 0.001**
MDA (μ mol/L) - Copper (μ mol/L)	0.776	< 0.001**
MDA (μ mol/L) - Zinc (μ mol/L)	-0.719	< 0.001**
MDA (μ mol/L) - Zinc/Copper ratio	-0.78	< 0.001**
Copper (μ mol/L) - Zinc/Copper ratio	-0.915	< 0.001**
Copper (μ mol/L) - Zinc (μ mol/L)	-0.767	< 0.001**
Zinc (μ mol/L) - Zinc/Copper ratio	-0.941	< 0.001**

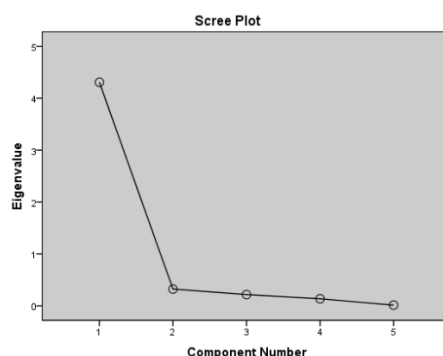
To explore latent structure, principal component analysis (PCA) was conducted. A single principal component (PC1) explained 86% of the total variance, with uniformly high loadings across all markers (Supplementary Table 4, Figure 3), reflecting shared variance. However, inclusion of

PC1 in regression models yielded unstable estimates, limiting interpretability. Consequently, discriminant function analysis (DFA) was pursued to retain the discriminative value of individual biomarkers while accounting for multicollinearity.

Table 4: Principal Component Analysis (PCA) loadings and explained variance for trace elements and oxidative stress biomarkers

Variables	component loading		
8-iso-Postaglandin factor 2 α (pg/mL)	0.939		
Malondialdehyde (μ mol/L)	0.879		
Copper (μ mol/L)	0.932		
Zinc (μ mol/L)	-0.913		
Zinc/Copper ratio	-0.974		
Component	Eigenvalue	% Variance explained	Cumulative %
PC1	4.307	86.13	86.13

One principal component (PC1) was retained, explaining over 86.13% of the total variance. Strong positive and negative loadings reflect collinearity among oxidative stress biomarkers and trace elements, justifying dimensionality reduction

**Figure 3: Scree plot showing only one major component (PC1) extracted from Principal Component Analysis (PCA).**

Values represent Pearson's correlation coefficients (r) and corresponding p -values (2-tailed) for associations between trace element levels and oxidative stress biomarkers. All correlations were statistically significant ($p < 0.001$), indicating strong interrelationships and suggesting substantial multicollinearity among the studied variables.

Discriminant function analysis of oxidative stress and trace element markers

DFA incorporating the five oxidative stress and trace element markers yielded a highly significant model (Wilks' $\lambda = 0.088$; $\chi^2 = 255.57$; $df = 10$; $p < 0.001$; Table 5). The Zn/Cu ratio had the strongest structure matrix loading (0.866), followed by 8-iso-PGF $_{2\alpha}$ and serum copper, indicating their prominent roles in distinguishing glycaemic status (see Figure 4).

Table 5. Summary of discriminant function analysis based on oxidative stress and trace element biomarkers

Variable	Coefficient	Structure Loading	Centroid	Apparent accuracy (%)	Cross-validated accuracy (%)
8-iso-PGF $_{2\alpha}$ (pg/mL)	-0.42	-0.733			
MDA (μ mol/L)	-0.253	-0.522			
Copper (μ mol/L)	0.328	-0.639			
Zinc (μ mol/L)	-0.306	0.626			
Zinc/Copper ratio	1.109	0.866			
Groups					
Healthy controls			2.848	97.5	96.4
Well-controlled diabetes mellitus			-0.005	80	77.6
Poorly controlled diabetes mellitus			-3.251	88.6	85.7
Overall accuracy				88.7	86.6

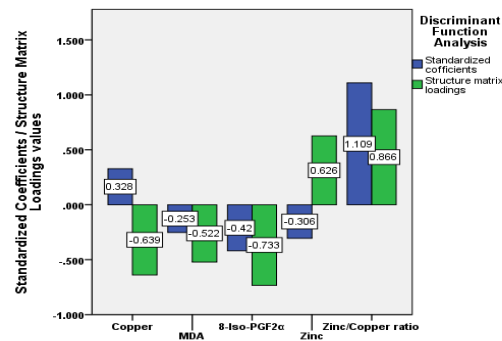


Figure 4: Standardised discriminant function coefficients and structure matrix loadings for trace elements and oxidative stress biomarkers

Coefficients indicate the relative contribution of each variable to group discrimination, while structure loadings represent the strength of correlation with the discriminant function. Zinc/Copper ratio showed the strongest discriminatory power, followed by 8-iso-PGF₂α and zinc.

Group centroids demonstrated clear separation: +2.848 (healthy controls), -0.005 (well-controlled diabetes), and -3.251 (poorly controlled diabetes),

supporting the model's discriminative ability (see Figure 5). Overall classification accuracy was 88.7%, with particularly high correct classification for healthy (97.5%) and poorly controlled diabetic (88.6%) participants. Leave-one-out cross-validation confirmed model robustness, yielding a comparable accuracy of 86.6%, reinforcing the utility of individual biomarker profiling in differentiating glycaemic phenotypes.

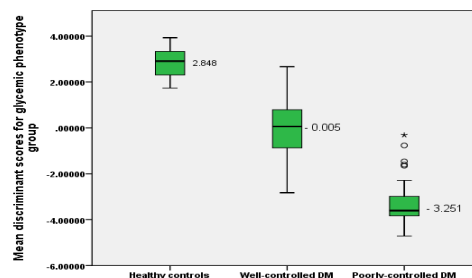


Figure 5: Glycaemic phenotype group centroids based on Discriminant Function Analysis (DFA)

Centroids reflect the mean discriminant scores for each group, illustrating clear separation across glycaemic states. Group positions along Function 1 indicate the degree and direction of discriminative influence. Canonical coefficients and structure loadings summarise variable contributions and correlations with the discriminant function, while group centroids indicate each phenotype's position. Classification accuracy (including cross-validation) reflects model performance.

Function 1 significantly discriminated among groups (Wilks' $\lambda = 0.088$, $\chi^2 = 255.57$, $df = 10$, $p < 0.001$).

Discussion

This study provides clear evidence that dysregulation of trace elements and oxidative

stress markers is closely associated with glycaemic control in type 2 diabetes mellitus (T2DM). Individuals with poorly controlled T2DM (HbA1c > 7.0%) demonstrated significantly higher serum copper, 8-iso-PGF₂α, and malondialdehyde (MDA) concentrations, alongside lower zinc levels and reduced Zn/Cu ratios, compared with well-controlled patients and healthy controls. Importantly, these associations persisted after adjustment for age, sex, body mass index, waist circumference, blood pressure, and diabetes duration, indicating an independent relationship not fully explained by these confounders.

Age, adiposity (particularly waist circumference), and hypertension are well-established contributors to insulin resistance, chronic low-grade inflammation, and reactive oxygen species (ROS)

production. Visceral fat accumulation promotes oxidative stress via increased free fatty acid flux, mitochondrial dysfunction, and inflammatory cytokine release (28), while elevated blood pressure reflects endothelial dysfunction and vascular oxidative stress, factors that may amplify lipid peroxidation and alter trace element homeostasis in poorly controlled T2DM participants. Nevertheless, the adjusted analyses indicate that poor glycaemic control exerts an additional, significant influence on these biomarkers.

Chronic hyperglycaemia, as reflected by HbA1c and plasma glucose, appears central to these alterations, promoting ROS overproduction through glucose autooxidation, advanced glycation end-product formation, protein kinase C activation, polyol and hexosamine pathways, and mitochondrial dysfunction (29), processes that likely drive the observed progressive rise in lipid peroxidation markers. Although trace element disturbances persisted after adjustment for HbA1c and plasma glucose, suggesting possible bidirectional influences, elevated copper may exacerbate oxidative damage via redox cycling, whereas zinc deficiency could impair antioxidant defences and insulin signalling.

The present findings are consistent with previous reports across diverse populations. The graded rise in lipid peroxidation markers across glycaemic categories reflects increasing oxidative burden with deteriorating glycaemic control. Elevated 8-iso-PGF₂α and MDA levels align with earlier studies identifying these markers as reliable indicators of lipid peroxidation and endothelial dysfunction, key processes implicated in diabetic vascular complications (13, 30). Notably, the progressive increase in 8-iso-PGF₂α across glycaemic phenotypes extends observations from both high-income settings and African cohorts, where similar elevations have been linked to microvascular damage and heightened cardiovascular risk in T2DM (31).

Similarly, reduced serum zinc levels, lower Zn/Cu ratios, and elevated copper levels in poorly controlled patients corroborate findings from previous global studies (32, 33), including those conducted in African populations (34). These trace element imbalances may contribute to oxidative stress amplification, impaired insulin action, and β-cell dysfunction, thereby exacerbating metabolic dysregulation (9, 10).

Correlation analyses demonstrated strong interrelationships among oxidative stress markers and trace elements, indicating substantial biological interdependence. In particular, 8-iso-

PGF₂α showed close associations with copper and the Zn/Cu ratio, while the Zn/Cu ratio was tightly linked to both zinc and copper levels. These patterns reflect shared regulatory pathways in redox balance and trace element homeostasis and highlight the potential for multicollinearity among these variables.

To address multicollinearity among interrelated markers, discriminant function analysis (DFA) and principal component analysis (PCA) were employed. DFA achieved high classification accuracy across glycaemic categories and identified the Zn/Cu ratio and 8-iso-PGF₂α as the most informative discriminative variables, underscoring their relevance beyond traditional cardiometabolic risk factors. PCA further confirmed shared variance among oxidative stress and trace element indices, supporting their close biological coupling rather than independent behaviour.

By stratifying participants according to glycaemic control rather than relying on a binary diabetic–non-diabetic comparison, this study provides a more nuanced view of metabolic deterioration in T2DM. This approach is particularly relevant for sub-Saharan African settings, where early risk stratification using affordable biomarkers remains limited. The Zn/Cu ratio emerged as a promising adjunct marker, given its strong discriminatory capacity, biological plausibility, and cost-effectiveness. Measurement of serum zinc and copper using flame atomic absorption spectrophotometry is well established, requires minimal sample volume, and is substantially less costly than advanced analytical techniques. As the ratio is derived from routinely measured parameters, it can be integrated into existing laboratory workflows with minimal additional analytical burden, provided appropriate quality assurance measures are in place.

Emerging evidence also suggests that modulation of zinc and copper status may contribute to improved redox balance; however, such therapeutic implications require confirmation through well-designed longitudinal and interventional studies (35, 36, 37).

Study Limitations

This study's cross-sectional design precludes causal inference, limiting conclusions regarding temporality between glycaemic control, oxidative stress, and zinc–copper imbalance. Prospective and interventional studies are therefore required to determine whether correction of trace element dysregulation improves glycaemic or oxidative outcomes and to elucidate underlying redox signalling mechanisms. Although participants with overt renal disease and those using mineral supplements were excluded to reduce confounding, subtle alterations in renal handling of zinc and copper, such as those related to early diabetic nephropathy or hyperglycaemia-induced hyperfiltration, cannot be completely excluded and may have influenced circulating trace element levels.

Conclusion

In conclusion, this study demonstrates an independent association between poor glycaemic control, oxidative stress, and zinc–copper dysregulation. The Zn/Cu ratio's superior discriminatory capacity positions it as a cost-effective biomarker to complement HbA1c in metabolic risk stratification, rather than replacing standard glycaemic indices. These findings are particularly relevant to low- and middle-income settings, offering an accessible adjunctive strategy for trace element profiling and diabetes management in resource-constrained environments.

List of Abbreviations

8-iso-PGF₂α: 8-Iso-Prostaglandin F₂ Alpha
AAS: Atomic Absorption Spectrophotometry
ADA: American Diabetes Association
ANOVA: Analysis of Variance
BMI: Body Mass Index
BUHREC: Babcock University Health Research Ethics Committee
DFA: Discriminant Function Analysis
ELISA: Enzyme-Linked Immunosorbent Assay
FPG: Fasting Plasma Glucose
HbA1c: Glycated Haemoglobin
MANCOVA: Multivariate Analysis of Covariance
MDA: Malondialdehyde
PCA: Principal Component Analysis
ROS: Reactive Oxygen Species
SOD: Superoxide Dismutase
T2DM: Type 2 Diabetes Mellitus
TBARS: Thiobarbituric Acid Reactive Substances

Declarations

Ethical Approval and Consent to Participate

This study received ethical approval from the Babcock University Health Research Ethics Committee (BUHREC), Ilisan-Remo, Ogun State, Nigeria (Reference Number: BUHREC 967/24). Written informed consent was obtained from all participants before enrolment. The study protocol adheres to the principles of the 1975 Declaration of Helsinki. To ensure participant anonymity, a barcode-based system was implemented, and all data were managed with strict confidentiality. The data collected were used exclusively for this study.

Consent for publication

Not applicable.

Availability of data and materials

The authors confirm that the data supporting the findings of this study is available within the article and its supplementary information files.

Competing interests

The authors declare no competing interests.

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Authors' contributions

NPC conceived the study, interpreted the data, drafted and reviewed the manuscript. CTJ performed the laboratory procedures, statistical analysis, and reviewed the manuscript. OMY performed the laboratory procedures, statistical analysis, and reviewed the manuscript. AAA contributed to data interpretation, drafting and reviewing the manuscript. USS contributed to statistical analysis, data interpretation, and manuscript editing. All authors approve the final version for submission.

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