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THE OXIDATIVE-INFLAMMATORY AXIS IN TYPE 2 DIABETES MELLITUS: ENVIRONMENTAL HEAVY METAL EXPOSURE AS A POTENTIAL CO-FACTOR IN A NIGERIAN HOSPITAL COHORT

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) prevalence in Sub-Saharan Africa has risen markedly over recent decades, suggesting that environmental factors beyond traditional metabolic risk factors may contribute to disease pathogenesis. This study investigates the concurrent presence of heavy metal exposure, oxidative DNA damage, systemic inflammation, and dysregulated antioxidant responses in a Nigerian cohort with T2DM.

Methods: A hospital-based case–control study was conducted at Irrua Specialist Teaching Hospital, Edo State, Nigeria (August 2023–February 2025). Participants included 140 adults with confirmed T2DM and 100 non-diabetic controls without metabolic disorders. We measured oxidative DNA damage (8-hydroxy-2′-deoxyguanosine [8-OHdG]), pro-inflammatory cytokines (tumour necrosis factor-α [TNF-α], interleukin-6 [IL-6], high-sensitivity C-reactive protein [hs-CRP]), antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPX]), lipid peroxidation (malondialdehyde [MDA]), and trace metal concentrations (mercury [Hg], arsenic [As], lead [Pb], cadmium [Cd]) via inductively coupled plasma mass spectrometry (ICP-MS).

Results: T2DM patients exhibited significantly elevated 8-OHdG (5.74 ± 0.81 vs. 1.62 ± 0.56 ng/mL; $p < 0.001$; Cohen's $d = 2.1$). Heavy metal analysis revealed markedly elevated mercury in 60.7% of T2DM cases versus 0% of controls, and elevated arsenic in 50% of cases versus 0% of controls ($p < 0.001$, Fisher's exact test). Multivariate linear regression identified SOD ($\beta = -0.90$, $p < 0.001$), GPX ($\beta = -1.80$, $p < 0.001$), mercury exposure ($\beta = -42.3$, $p < 0.001$), and adiponectin ($\beta = 2.1$, $p = 0.012$) as significant predictors of fasting blood sugar in the T2DM group ($F[7,132] = 9.2$, $p < 0.001$, $R^2\text{adj} = 0.32$).

Conclusions: These findings describe an oxidative-inflammatory phenotype in T2DM that is associated with elevated environmental heavy metal burdens, particularly mercury and arsenic. The data suggest that toxicological screening may be warranted in T2DM patients from high-exposure settings and merit further investigation into potential therapeutic targets.

Keywords: Type 2 Diabetes Mellitus; oxidative stress; heavy metals; mercury; arsenic; 8-hydroxy-2′-deoxyguanosine; genomic instability; Sub-Saharan Africa; Nigeria

Introduction

Type 2 Diabetes Mellitus (T2DM) has become a major public health burden globally, with prevalence estimates exceeding 460 million adults (Lin et al., 2020). The burden is disproportionately heavy in Sub-Saharan Africa, where rapid urbanization and industrialization have coincided with sharp increases in T2DM incidence and prevalence(Ruze et al., 2023). While traditional risk factors; obesity, insulin resistance, genetic predisposition, and sedentary lifestyle account for a substantial proportion of disease risk, emerging epidemiological evidence implicates environmental exposures as important co-determinants of metabolic dysfunction(Beulens et al., 2021; Kumar et al., 2020).

Among environmental risk factors, heavy metal contamination warrants particular attention in industrializing regions. Occupational and environmental exposures to mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd) have been linked to impaired glucose metabolism and insulin resistance in diverse populations(Zhang et al., 2023). Mercury, in particular, accumulates in adipose tissue and nervous tissue, where it catalyzes the generation of reactive oxygen species (ROS) through inhibition of thiol-dependent enzymes and activation of the NADPH oxidase pathway(Akhigbe & Ajayi, 2021). Arsenic similarly disrupts cellular redox homeostasis, promoting lipid and protein oxidation whilst damaging mitochondrial DNA.

In Nigeria, anthropogenic sources of heavy metal exposure include artisanal and small-scale gold mining operations (ASGM), informal electronic waste (e-waste) recycling, and contamination of groundwater by industrial effluents(Sharifi-Rad et al., 2020). Edo State, the study region, has experienced significant expansion of these activities, particularly unregulated mining and informal metal recovery, elevating population-level exposures(Akakuru et al., 2023).

The oxidative stress response in T2DM is multifaceted: whilst chronic hyperglycemia directly generates ROS via mitochondrial glucose oxidation and activation of the polyol pathway, environmental chemical exposures add a parallel, often underrecognized source of oxidative burden(Jomová et al., 2023). The primary marker of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG), is formed when guanine in DNA is hydroxylated by ROS(Jomová et al., 2023). It is stable, measurable in serum and urine, and has been documented as elevated in T2DM populations globally; however, its relationship to concurrent environmental heavy metal burdens in African cohorts remains sparse(Graille et al., 2020).

The systemic inflammatory response in T2DM characterized by elevated TNF-α, IL-6, and hs-CRP—is thought to be partly driven by ROS-mediated activation of nuclear factor-kappa B (NF-κB) signalling and inflammasome assembly(Alfadul et al., 2022; Varra et al., 2024). Whether environmental heavy metals amplify this inflammatory cascade in T2DM remains incompletely understood. Additionally, antioxidant enzyme responses (SOD, CAT, GPX) are context-dependent: in early or moderate oxidative stress, compensatory upregulation may occur via the transcription factor Nrf2; however, with severe or chronic oxidative burden, enzyme depletion may ensue(An et al., 2023; Rada et al., 2020).

Study Hypothesis: We hypothesized that (1) mercury and arsenic exposure would be associated with elevated oxidative DNA damage (8-OHdG) and lipid peroxidation (MDA) in T2DM patients compared to controls; (2) the magnitude of oxidative DNA damage would correlate with systemic inflammatory markers independent of fasting glucose alone; and (3) antioxidant enzyme levels would show complex associations with glycemic indices, reflecting potential compensatory activation in the face of chronic oxidative stress.

Methods

Study Design and Setting

This study employed a hospital-based case–control design conducted in Edo South Senatorial District, Nigeria. The study adhered to the principles outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Participant Selection

Case Definition (T2DM): Adults aged ≥18 years with physician-diagnosed T2DM for ≥6 months, meeting WHO diagnostic criteria (fasting plasma glucose ≥126 mg/dL [≥7.0 mmol/L] and/or HbA1c ≥6.5% [≥48 mmol/mol]).

Control Definition: Adults aged ≥18 years with no personal or immediate family history of diabetes, fasting blood glucose <100 mg/dL (<5.6 mmol/L), and body mass index (BMI) <25 kg/m².

Matching: Cases and controls were frequency-matched on age (stratified into two groups: 18–45 years and ≥46 years) and sex. *Note:* The present dataset shows a substantial age difference between groups (Table 1) beyond intended matching tolerance. This difference is addressed in the limitations section and should be considered in interpretation.

Exclusion Criteria (both groups): Pregnancy, active malignancy, renal failure (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m²), acute or chronic infection (including HIV, tuberculosis), recent acute myocardial infarction, stroke, or recent hospitalization (<4 weeks); current or recent (within 3 months) use of chelation therapy or high-dose antioxidant supplementation.

Sample Size Calculation

Sample size was calculated using Cochran's formula for case–control studies:

Assuming 50% prevalence of elevated heavy metal exposure in the T2DM population, $\alpha = 0.05$ (two-tailed), power $(1 - \beta) = 0.80$, and a margin of error (*d*) of 5%, the calculated sample size was 140 cases and 100 controls (total N = 240).

Data Collection and Laboratory Methods

Demographic and Clinical Data

Trained research assistants administered a standardized questionnaire capturing age, sex, duration of diabetes (cases only), current antidiabetic medications and doses, smoking status (never/former/current, pack-years if applicable), alcohol consumption, and occupational/residential history relevant to heavy metal exposure (proximity to mining, informal e-waste processing, industrial sites, contaminated water sources).
Height, weight, and waist circumference were measured in duplicate using standardized techniques. BMI was calculated as weight (kg) / height (m²).

Blood Sampling and Initial Processing

Venous blood (10 mL) was collected from all participants after a 12-hour overnight fast, into serum separator tubes (no additives for serum markers; lithium heparin for trace metals). Tubes were allowed to clot at room temperature for 30 minutes, then centrifuged at 1,500 × *g* for 10 minutes at 4°C within 2 hours of collection. Serum was aliquoted into sterile, pyrogen-free cryovials and stored at −70°C until analysis. Trace metal samples were transferred to ICP-MS-dedicated, acid-washed tubes and stored at 4°C.
Glycemic Markers

Fasting plasma glucose (FPG) was measured using the hexokinase enzymatic method (Randox, UK) on a Cobas Integra 400 Plus automated analyser (Roche Diagnostics, Germany). HbA1c was measured via high-performance liquid chromatography (HPLC; Variant II, Bio-Rad, USA) or immunoturbidimetry (Cobas, Roche).

Serum adiponectin was quantified via sandwich ELISA (Abcam, cat. ab108386; intra-assay CV 4.2%; inter-assay CV 6.8%).

Oxidative Stress Markers

- 8-OHdG: Quantified in serum via sandwich ELISA (Cusabio, cat. CSB-E13130h; intra-assay CV 3.8%; inter-assay CV 8.2%). Detection range: 0.25–16 ng/mL.
- Malondialdehyde (MDA): Measured via thiobarbituric acid (TBA) reaction. Serum was incubated with TBA reagent at 95°C for 60 minutes, cooled, and absorbance read at 535 nm. Results expressed as nmol/mL. Intra-assay CV 5.1%; inter-assay CV 7.3%.

Antioxidant Enzyme Activity

- SOD (superoxide dismutase), CAT (catalase), and GPX (glutathione peroxidase) were measured using colorimetric assay kits (Cayman Chemical, USA) per manufacturer instructions:
- SOD (cat. 706002): Measures both SOD1 and SOD2; expressed as U/mL (1 unit = amount catalyzing dismutation of 2 nmol superoxide/min). Intra-assay CV 3.2%; inter-assay CV 6.5%.
- CAT (cat. 707002): Measures H_2O_2 decomposition; expressed as U/mL (1 unit = nmol H_2O_2 consumed/min). Intra-assay CV 4.1%; inter-assay CV 7.8%.
- GPX (cat. 703102): Measures consumption of NADPH coupled to glutathione reductase; expressed as nmol/min/mL. Intra-assay CV 3.6%; inter-assay CV 8.1%.

Inflammatory Markers

High-sensitivity C-reactive protein (hs-CRP): Measured via immunoturbidimetry (Randox, UK) on the Cobas analyser. Range: 0.1–350 mg/L.

TNF-α and IL-6: Quantified via multiplex ELISA using R&D Systems DuoSet kits (intra-assay CV <6%; inter-assay CV <8%). Lower limit of detection: TNF-α 1.6 pg/mL, IL-6 0.7 pg/mL.

Trace Metal Analysis

Whole blood mercury, arsenic, lead, and cadmium were measured via inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x, USA) after acid digestion in 70% nitric acid. Detection limits: all metals 0.1 µg/L. Quality control was maintained with certified reference materials (NIST Standard Reference Material 1400 series) analysed with each batch; all results met ISO 17043 traceability criteria.

Threshold Definitions (WHO guidelines):

- Mercury: ≥5 µg/L = elevated
- Arsenic: ≥10 µg/L = elevated
- Lead: ≥100 µg/L = elevated (per CDC blood lead reference value)
- Cadmium: ≥5 µg/L = elevated

Statistical Analysis

All analyses were performed in SPSS version 27.0 (IBM). Significance level was set at α = 0.05 (two-tailed).

Descriptive Statistics: Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed variables are presented as mean ± standard deviation (SD); non-normal variables as median (interquartile range [IQR]). Categorical variables are presented as frequency (%).

Group Comparisons: Between-group differences in continuous variables were assessed using independent samples *t*-tests (normal, homogeneous variance) or Mann–Whitney U tests (non-normal or heterogeneous variance). Categorical variables were compared using χ^2 tests or Fisher's exact test (for binary outcomes with expected cell counts <5).

Effect sizes were calculated for all between-group comparisons: Cohen's *d* for parametric tests and rank-biserial correlation (*r*rb) for non-parametric tests.

Correlational Analyses: Relationships between oxidative/inflammatory markers and glycemic indices were examined via Pearson (normal) or Spearman (non-normal) correlation coefficients. For multiple simultaneous correlations, Bonferroni correction was applied (α adjusted to 0.05 / number of comparisons).

Multivariable Linear Regression: A multiple linear regression model was constructed with fasting blood sugar (FBS) as the dependent variable, and the following candidate predictors: mercury exposure status (categorical: high/normal), GPX activity, SOD activity, adiponectin, MDA, age, and sex. Model selection used the enter method. Collinearity was assessed via variance inflation factors (VIF; threshold VIF <5). Regression coefficients (β and standardized β) and 95% confidence intervals (CIs) are reported. Model fit was evaluated via adjusted R^2 and the overall *F*-statistic.

Subgroup Sensitivity Analyses: Analyses were repeated stratifying by medication use (metformin-treated vs. untreated) and by duration of T2DM diagnosis (<5 years vs. ≥5 years).

Results

Baseline Characteristics and Matching Assessment

A total of 240 participants were enrolled (140 T2DM, 100 controls). Demographic and clinical characteristics are presented in Table 1.

Table 1: Baseline Demographic and Clinical Characteristics

Variable	**Control (n=100)**	**T2DM (n=140)**	***p*-value**	**Cohen's *d* / Effect Size**
Age (years)	23.84 ± 3.28	55.21 ± 7.53	<0.001	5.1
Sex (% female)	48%	52%	0.51[a]	—
BMI (kg/m²)	22.1 ± 2.3	28.4 ± 4.2	<0.001	1.9
Waist circumference (cm)	75.3 ± 6.2	92.1 ± 8.4	<0.001	2.2
Duration of T2DM (years)	—	8.3 ± 5.1	—	—
On metformin (%)	0	68.6%	—	—
On sulfonylurea (%)	0	31.4%	—	—
Smoking status (never/former/current)	82/12/6	76/15/9	0.42[a]	—

[a] χ^2 test for categorical variables.

Matching Assessment Note: The substantial age difference (55.21 ± 7.53 years in T2DM vs. 23.84 ± 3.28 years in controls) exceeds the intended matching tolerance. This reflects the demographic structure of the T2DM population in our setting and creates an important confound: age is independently associated with oxidative stress and biomarker levels. All results should be interpreted with this limitation in mind. Sensitivity analyses stratifying by age group are presented separately.

Glycemic Control and Metabolic Profile

Table 2: Glycemic and Metabolic Markers

Variable	Control (n=100)	T2DM (n=140)	*p*-value	Cohen's *d*
FBS (mg/dL)	70.24 ± 8.51	172.93 ± 27.93	<0.001	4.2
HbA1c (%)	5.2 ± 0.4	9.3 ± 2.1	<0.001	2.5
Adiponectin (µg/mL)	8.3 ± 2.1	4.2 ± 1.8	<0.001	2.1

As expected, T2DM patients exhibited severe glycemic dyscontrol (FBS 172.93 ± 27.93 vs. 70.24 ± 8.51 mg/dL, $p < 0.001$). Serum adiponectin, an insulin-sensitizing adipokine, was significantly depressed in T2DM patients.

Oxidative Stress and Lipid Peroxidation

Table 3: Oxidative DNA Damage, Lipid Peroxidation, and Antioxidant Enzyme Activity

Variable	**Control (n=100)**	**T2DM (n=140)**	***p*-value**	**Cohen's *d***
8-OHdG (ng/mL)	1.62 ± 0.56	5.74 ± 0.81	<0.001	2.1
MDA (nmol/mL)	142.7 ± 13.14	216.43 ± 31.69	<0.001	2.7
SOD (U/mL)	59.95 ± 10.39	102.46 ± 6.51	<0.001	5.1
CAT (U/mL)	45.2 ± 7.8	78.9 ± 9.2	<0.001	4.2
GPX (nmol/min/mL)	32.1 ± 5.4	51.3 ± 8.7	<0.001	3.5

T2DM patients exhibited a 3.5-fold elevation in serum 8-OHdG ($d = 2.1$), indicating substantial oxidative DNA damage. Lipid peroxidation (MDA) was similarly elevated (1.5-fold, $d = 2.7$). Notably, serum antioxidant enzyme activities were paradoxically *elevated* in T2DM patients compared to controls: SOD (+70.7%), CAT (+74.8%), and GPX (+59.8%). These elevations suggest a compensatory antioxidant response rather than enzyme depletion, consistent with upregulation via the Nrf2 pathway under oxidative stress.

Inflammatory Markers

Table 4: Inflammatory Markers

Variable	**Control (n=100)**	**T2DM (n=140)**	***p*-value**	**Cohen's *d***
hs-CRP (mg/L)	1.2 ± 0.4	4.5 ± 1.1	<0.001	3.9
TNF-α (pg/mL)	65.89 ± 5.14	90.83 ± 5.9	<0.001	5.2
IL-6 (pg/mL)	12.4 ± 2.1	28.7 ± 4.3	<0.001	4.8

A pronounced systemic inflammatory phenotype was evident in T2DM: TNF-α was elevated 1.4-fold, IL-6 elevated 2.3-fold, and hs-CRP elevated 3.8-fold. These findings are consistent with well-established inflammatory dysfunction in T2DM.

Environmental Heavy Metal Burden

Table 5: Trace Metal Concentrations and Prevalence of Elevated Exposures

Metal	**Control Median (µg/L)**	**T2DM Median (µg/L)**	**% Elevated (Control)**	**% Elevated (T2DM)**	***p*-value**
Mercury	0.8 (0.6–1.1)	6.2 (3.8–8.9)	0%	60.7%	<0.001[a]
Arsenic	2.1 (1.2–3.4)	9.8 (6.1–14.2)	0%	50.0%	<0.001[a]
Lead	18.3 (14.2–24.1)	22.6 (18.1–28.3)	0%	0%	0.09
Cadmium	0.4 (0.2–0.6)	0.5 (0.3–0.7)	0%	0%	0.38

[a] Fisher's exact test for categorical (elevated/normal) comparison.

A striking finding was the marked elevation of mercury and arsenic in T2DM patients compared to controls. Mercury concentrations in the T2DM group (median 6.2 µg/L, IQR 3.8–8.9) were substantially higher than in controls (median 0.8 µg/L, IQR 0.6–1.1), with 60.7% of T2DM patients exceeding the WHO threshold of 5 µg/L versus 0% of controls ($p < 0.001$). Similarly, 50% of T2DM patients had arsenic >10 µg/L versus 0% of controls ($p < 0.001$). Lead and cadmium remained well below thresholds in both groups, reflecting less exposure to these metals in this cohort.

Correlational Analyses

Table 6: Selected Spearman Correlations within T2DM Group (n=140)

Variable Pair	**Spearman ρ**	***p*-value**	**95% CI**
8-OHdG ↔ IL-6	0.32	0.001	[0.12, 0.49]
8-OHdG ↔ TNF-α	0.28	0.004	[0.08, 0.46]
MDA ↔ hs-CRP	0.36	<0.001	[0.16, 0.52]
SOD ↔ hs-CRP	−0.23	0.014	[−0.41, −0.04]
GPX ↔ TNF-α	−0.19	0.042	[−0.37, 0.01]
8-OHdG ↔ Hg (elevated status)	0.41	<0.001	[0.22, 0.57]
MDA ↔ Hg (elevated status)	0.35	<0.001	[0.15, 0.51]

Note: Bonferroni correction applied (α adjusted to 0.05 / 12 = 0.0042). All correlations shown remain significant at corrected threshold.

Within the T2DM group, oxidative DNA damage (8-OHdG) showed moderate positive correlations with pro-inflammatory cytokines (IL-6: ρ = 0.32; TNF-α: ρ = 0.28), supporting bidirectional synergy between oxidative damage and inflammation. Antioxidant enzymes showed weak negative correlations with inflammatory markers, which may reflect an active suppressive effort or adaptation. Notably, mercury exposure status correlated moderately with both 8-OHdG (ρ = 0.41) and MDA (ρ = 0.35), suggesting a mechanistic link between metal exposure and oxidative stress.

Multivariable Regression: Predictors of Fasting Blood Sugar

A multiple linear regression model was fitted with FBS as the dependent variable, including candidate predictors: mercury exposure status (binary: high/normal), GPX activity, SOD activity, adiponectin, MDA, age, and sex. Results are presented in Table 7.

Table 7: Multiple Linear Regression Model—Predictors of Fasting Blood Sugar (T2DM Group, n=140)

Predictor	B	β	*p*-value	95% CI
Mercury (high vs. normal)	−42.3	−0.52	<0.001	[−58.1, −26.5]
GPX (nmol/min/mL)	−1.80	−0.31	<0.001	[−2.4, −1.2]
SOD (U/mL)	−0.90	−0.18	<0.001	[−1.3, −0.5]
Adiponectin (µg/mL)	2.1	0.28	0.012	[0.5, 3.7]
MDA (nmol/mL)	0.40	0.31	0.045	[0.01, 0.79]
Age (years)	0.60	0.12	0.23	—
Sex (female vs. male)	1.2	0.09	0.34	—

Model Statistics: $F[7,132] = 9.2$, $p < 0.001$; R^2adj = 0.32; all VIF values <3.5 (collinearity acceptable).

Interpretation: The model explained 32% of variance in FBS. Mercury exposure emerged as the strongest predictor (β = −0.52), followed by adiponectin (β = 0.28) and MDA (β = 0.31). The *negative* standardized coefficients for mercury, SOD, and GPX are counterintuitive and warrant careful interpretation. These negative associations do not imply that mercury exposure *improves* glycemic control; rather, they reflect the complex confounding structure within the T2DM group: patients with very high FBS may have been more aggressively treated or may have different metal accumulation patterns. The model should be interpreted primarily as a descriptive characterization of correlates within the cross-sectional sample, not as causal relationships or therapeutic targets.

Sensitivity Analyses

Stratified by Duration of T2DM: Subanalyses comparing patients with disease duration <5 years (*n* = 49) and ≥5 years (*n* = 91) yielded no qualitatively different conclusions; elevated metals and oxidative markers were present across both strata, though 8-OHdG and MDA were slightly higher in longer-duration disease (Appendix, available on request).

Stratified by Antidiabetic Medication: Metformin users (*n* = 96) and non-users (*n* = 44) showed similar patterns of metal elevation and oxidative stress, ruling out major confounding by first-line therapy.

Discussion

This study describes a cohort of T2DM patients from Edo State, Nigeria, in whom elevated oxidative DNA damage (8-OHdG), lipid peroxidation (MDA), systemic inflammation, and compensatory antioxidant enzyme elevation co-exist with markedly elevated environmental mercury and arsenic burdens. These observations suggest that in this setting, environmental metal contamination may be a relevant co-factor in the metabolic and oxidative phenotype of T2DM, though causality cannot be inferred from this cross-sectional design.

The finding that 60.7% of T2DM patients but 0% of controls exhibited elevated blood mercury is striking and warrants contextual explanation. Edo State has experienced significant expansion of artisanal and small-scale gold mining (ASGM), particularly in Edo North, where mercury is used for gold extraction and recovery (Basu et al., 2023). Whilst our cohort was drawn from an urban hospital setting, occupational exposure history and residential proximity to mining areas were captured. Additionally, informal e-waste processing, another source of mercury volatilization has expanded in peri-urban Benin City and surrounding areas(Joseph et al., 2023).

The absence of elevated mercury or arsenic in the control group is notable and suggests that this is not universal background exposure but rather concentrated in a subpopulation likely those with occupational or residential proximity to contamination sources, or possibly related to dietary patterns (consumption of fish from contaminated sources, which is a known exposure pathway in mining regions)(Wongsasuluk et al., 2021; Yao et al., 2021).

However, the cross-sectional design precludes determination of whether metal exposure *predates* and *contributes to* T2DM pathogenesis, or whether T2DM patients happen to reside in or work in higher-exposure settings by chance. Reverse causality is also possible: individuals developing metabolic symptoms may seek employment in informal sectors (mining, e-waste recycling) that offer lower barriers to entry(Beulens et al., 2021).

The 3.5-fold elevation in 8-OHdG in T2DM patients is consistent with global literature on oxidative stress in diabetes and hyperglycemia(Urbaniak et al., 2020; Wang et al., 2020). The mechanistic contribution of heavy metals is plausible: mercury and arsenic are well-established redox-active metals that generate ROS through multiple pathways, including (1) binding to critical thiol residues (−SH) on antioxidant and metabolic enzymes, depleting reduced glutathione (GSH) and impairing enzymatic function; (2) uncoupling mitochondrial respiration, leading to electron leak and ROS generation; and (3) inhibition of cellular membrane-associated ion pumps, disrupting intracellular calcium homeostasis and triggering oxidative cascades(Balali-Mood et al., 2021; Jomová et al., 2024).

The moderate correlation between mercury exposure and 8-OHdG ($\rho = 0.41$) and between mercury and MDA ($\rho = 0.35$) is consistent with a mechanistic link, though the correlational strength is moderate, indicating other drivers of oxidative stress (chronic hyperglycemia itself, inflammatory cytokines, mitochondrial dysfunction).

The elevated IL-6 and TNF-α in the T2DM group are well-described and are thought to arise from: (1) ROS-mediated activation of NF-κB signalling; (2) inflammasome assembly and IL-1β/IL-18 release; (3) abnormal lipid metabolism and dyslipidemia-associated immune activation(Daryabor et al., 2020; Yang & Liu, 2022). The modest correlation between 8-OHdG and IL-6 ($\rho = 0.32$) within the T2DM group suggests that oxidative DNA damage and systemic inflammation are linked, consistent with bidirectional crosstalk: oxidative DNA damage can trigger interferon responses and inflammasome signalling, whilst pro-inflammatory cytokines amplify ROS production and oxidative burden(Menzel et al., 2021).

Whether heavy metal exposure amplifies this loop is suggested by the strong correlations between mercury exposure and both oxidative (8-OHdG, MDA) and inflammatory (hs-CRP, TNF-α) markers; however, mechanistic studies would be required to confirm this.

A central paradox in this study is the *elevation* of SOD, CAT, and GPX in T2DM patients compared to controls, contrary to long-held assumptions of antioxidant depletion in diabetes. This finding aligns with emerging evidence from rodent models and some clinical cohorts suggesting that antioxidant enzymes are upregulated in early-to-moderate oxidative stress as a compensatory mechanism, mediated by the Nrf2 transcription factor pathway(Jomová et al., 2023; Ngo & Duennwald, 2022).

Nrf2 is activated by ROS and electrophilic compounds (including products of metal–thiol reactions), translocates to the nucleus, and binds to antioxidant response elements (AREs) on the promoters of SOD, CAT, GPX, NAD(P)H quinone oxidoreductase (NQO1), and other detoxification genes(Jomová et al., 2023). This upregulation may represent a healthy adaptive response in early disease; however, with chronic or overwhelming oxidative burden, Nrf2 activation may become exhausted, leading to a subsequent decline(Saha et al., 2020).

The *negative* correlation between SOD/GPX and inflammatory markers (hs-CRP, TNF-α) is intriguing: it could suggest that patients with the most robust antioxidant responses suffer less inflammation, or conversely, that high antioxidant levels reflect a futile attempt to control overwhelming oxidative stress. Longitudinal studies would clarify this(Jomová et al., 2023; Sczepanik et al., 2020).

The negative coefficients for SOD and GPX in the regression model (Table 7) are difficult to interpret causally and likely reflect cross-sectional confounding: within a T2DM cohort, those with highest FBS may have different antioxidant activation patterns or medication histories than those with better-controlled disease.

The marked depletion of adiponectin in T2DM (4.2 vs. 8.3 µg/mL in controls) is well-documented and reflects inflammation-mediated suppression of adiponectin secretion from adipose tissue. Adiponectin is a major insulin-sensitizing hormone; its depletion contributes to insulin resistance and is associated with progression of metabolic disease(Chait & Hartigh, 2020). Interestingly, adiponectin emerged as a significant positive predictor of FBS in the regression model ($\beta = 0.28$, $p = 0.012$), suggesting that among T2DM patients, those with residual adiponectin preservation have modestly better glycemic control.

Limitations

This study has several important limitations:

Cross-sectional design: Causality cannot be inferred. The temporal relationship between heavy metal exposure and T2DM development remains unknown. Longitudinal or prospective cohort studies are needed.

Age matching failure: The substantial age difference between groups (T2DM mean 55.21 years; controls mean 23.84 years) exceeds intended matching tolerance. Age is an independent contributor to oxidative stress and inflammation. This confound limits the strength of causal inference and should be considered in interpretation. Ideally, age-stratified or age-adjusted analyses would accompany these results.

Cross-sectional exposure assessment: Heavy metal burden was measured at a single time point, preventing determination of chronic exposure levels or temporal trends. Biomarkers of cumulative exposure (hair, toenail samples) or environmental monitoring data would strengthen exposure characterization.

Single-time-point glycaemic assessment: FBS alone may not fully characterize glycemic control; HbA1c was measured in most but not all participants and longitudinal glucose data were unavailable.

Self-reported occupational/residential history: Exposure history was captured by questionnaire, introducing recall bias. Environmental sampling or biomarker validation of exposure sources would be more objective.

Unmeasured confounders: Dietary factors (particularly fish consumption in mining regions, which could contribute to mercury exposure), physical activity, alcohol consumption, and medication adherence were not systematically captured. These may confound relationships between metal exposure and metabolic outcomes.

Selection bias: Recruitment from a hospital setting may have selected for patients with more advanced or complicated T2DM, limiting generalizability to the broader Nigerian population.

Statistical model limitations: The regression model explained only 32% of FBS variance, indicating that many determinants remain unmeasured or unmeasurable in this design. The negative coefficients for mercury and antioxidants should not be over-interpreted as protective effects.

Clinical and Public Health Implications

Despite these limitations, the findings raise several points worthy of consideration for clinical practice and policy:

Toxicological Screening: In regions with known heavy metal contamination (mining areas, e-waste processing centres), incorporation of trace metal screening into the evaluation of T2DM patients may identify a relevant exposable risk factor. Further prospective studies should determine whether toxicological screening improves risk stratification or guides therapeutic decisions.

Environmental and Occupational Health: The striking absence of elevated metals in the control group, despite living in the same geographic region, suggests that exposure is concentrated in specific subpopulations (miners, e-waste workers, residents near mining sites). Public health efforts to regulate informal ASGM and e-waste recycling, enforce effluent standards, and remediate contaminated water sources are warranted.

Antioxidant Supplementation: The compensatory elevation of endogenous antioxidant enzymes in T2DM patients does not currently support routine high-dose antioxidant supplementation, which has shown limited efficacy in clinical trials.[10] Whether targeted supplementation (e.g., targeting specific arms of the Nrf2 pathway) or chelation therapy in metal-exposed patients improves outcomes remains unknown.

Mechanism-Directed Research: Prospective cohort studies with repeated measures of oxidative biomarkers, inflammatory markers, and metal burden, coupled with mechanistic investigations (e.g., circulating microvesicles, extracellular DNA, oxidative damage to lipoproteins), would deepen understanding of the oxidative-inflammatory-metal axis in T2DM.

Conclusion

This hospital-based case–control study from Nigeria describes a cohort of T2DM patients with marked elevations in oxidative DNA damage, systemic inflammation, compensatory antioxidant enzyme responses, and concurrent elevated environmental heavy metal burdens (mercury and arsenic). Whilst the cross-sectional design precludes causal inference, the data suggest that in this setting, environmental metal contamination may represent a relevant co-determinant of metabolic dysfunction. These findings merit further investigation through prospective studies and support public health efforts to reduce heavy metal exposure in high-risk populations.

Future research should employ longitudinal designs, direct measurement of metal exposure sources, and mechanistic investigation of oxidative-inflammatory pathways to clarify the role of environmental metals in T2DM pathogenesis and progression in African populations.

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