

Journal of Molecular Science

www.jmolecularsci.com

ISSN:1000-9035

Oxidative Stress and Antioxidant Dynamics in Cancer, Diabetes Mellitus, Anemia, and cardiovascular diseases

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Article Information

Received: 23-08-2026

Revised: 04-09-2026

Accepted: 18-09-2026

Published: 06-10-2026

Keywords

Oxidative Stress, Reactive Oxygen Species, Antioxidant Defense, Cancer, Diabetes Mellitus, Anemia, Cardiovascular Disease, Biomarkers,

ABSTRACT

Background: Oxidative stress is known to play a role in the pathogenesis of many chronic diseases, and comparative quantitative assessment across disease categories is limited. This study was aimed at characterizing oxidative stress biomarker profile and antioxidant defense status in patients of cancer, diabetes mellitus (DM), anemia and cardiovascular diseases (CVD) in comparison to healthy individuals. **Methods:** The study design was cross sectional analytical and included the participants, 240 cases (60 each disease group) and 60 age matched healthy controls from the tertiary care hospitals. Standardized assays were used to measure the levels of plasma malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), F₂-isoprostanes, protein carbonyls, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and vitamin E, vitamin C and total antioxidant capacity (TAC). The severity of the disease was graded based on the clinical indices validated for the disease. **Results:** Disease groups had significantly higher oxidative damage biomarkers than controls ($p < 0.001$). Cancer patients exhibited the highest MDA levels (7.8 ± 2.1 vs. 3.2 ± 0.8 nmol/mL; $p < 0.001$), followed by anemia (6.4 ± 1.9), diabetes (5.9 ± 1.6), and CVD (5.5 ± 1.4). Antioxidant depletion was most pronounced in anemia (GSH: 3.8 ± 1.0 vs. 7.8 ± 1.4 μ mol/L; $p < 0.001$). Positive correlations were seen between the level of MDA and the disease severity index for all groups ($r = 0.54$ – 0.72 , all with $p < 0.01$). The cancer group had the highest oxidative stress index (OSI) (3.8 ± 1.2) compared with other groups ($p < 0.001$). The paradoxical hyperactivation of Nrf2 in cancer was revealed and its impaired activation in other diseases was also established. **Conclusions:** This study reveals different oxidative stress signatures in cancer, DM, anemia and CVD, the former exhibiting the highest oxidative stress burden, and the latter the greatest antioxidant depletion. Such disease-specific patterns indicate the need for precision redox medicine strategies instead of a one-size-fits-all approach to antioxidants.

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1. INTRODUCTION:

Non-communicable diseases (NCDs) contribute about 74% of all deaths worldwide, and cancer, diabetes mellitus, anaemia related diseases and cardiovascular diseases form the bulk of chronic diseases burdening morbidity and mortality (World Health Organization, 2024). Although clinically heterogeneous, they share a number of common molecular roots, with oxidative stress increasingly considered as a common pathophysiological mechanism of great importance (Liguori et al., 2023). Oxidative stress emerges when the balance between production of reactive oxygen

species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) and the capacity to neutralize them by enzymatic and non-enzymatic antioxidant defense systems is disturbed in favour of the pro-oxidant state (Sies et al., 2022). ROS play important functions in cellular signaling, host defense and metabolic regulation under physiologic conditions. Persistent increases in ROS, however, trigger a chain of events that lead to molecular damage of lipids, proteins, and nucleic acids, eventually causing damage to cellular integrity and function (Murphy, 2021).

Although many studies have focused on oxidative stress in specific disease groups, few quantitative studies have investigated oxidative stress in several chronic diseases with standardized panels of biomarkers. Other studies have been largely conducted in confined disease groups or with different types of assays, making comparisons across disease groups difficult. In addition, there is a lack of systematic characterization of the association between levels of oxidative stress biomarkers and disease severity in these major NCD groups. The endogenous antioxidant defense network includes non-enzymatic molecules such as reduced glutathione (GSH), vitamin E, vitamin C and coenzyme Q_{10} , as well as enzymatic scavengers such as superoxide dismutases (SOD1, SOD2, SOD3), catalase (CAT), glutathione peroxidases (GPx1–GPx4) (Forman et al., 2022). This defense network is regulated by the Nrf2/Keap1/ARE signaling axis, the master transcriptional regulator of this network (Tonelli et al., 2023). But the differential response of this system is not fully understood between disease categories.

ROS play a dual role in cancer biology; low and moderate levels can support cancer initiation by activation of proliferative signaling pathways, while high levels of ROS can lead to cancer cell death (Gorrini et al., 2023; Zhou et al., 2024). Overproduction of mitochondrial ROS is a key early event in diabetes, which leads to several harmful pathways (Brownlee, 2021; Forbes and Thorpe, 2023). Iron metabolism and oxidative stress are coupled in a two-way relationship in anemia (Papanikolaou and Pantopoulos, 2022). Oxidative stress plays a role in all phases of atherogenesis in the context of CVD (Holmes et al., 2023). Study Rationale: There is a mechanistic link between oxidative stress in all of these disease states and a dearth of comparative, quantitative data; thus, this cross-sectional analytical study was conducted with the goals of: (1) characterizing and comparing oxidative damage biomarker profiles for patients with cancer, DM, anemia and CVD versus healthy controls; (2) quantifying antioxidant defense depletion patterns; (3) determining correlations between oxidative stress markers and disease severity; and (4) identifying disease-specific oxidative

vulnerability signatures that could guide precision therapeutic strategies.

2. REVIEW OF LITERATURE:

2.1 Oxidative Stress in Cancer:

In the tumour microenvironment there is chronic oxidative stress, which has both pro- and anti-tumourigenic effects. Trachootham et al. 2021 have shown that cancer cells maintain a “Goldilocks zone” of ROS levels that are high enough to activate redox-sensitive pathways that promote cell proliferation such as NF- κ B, AP-1 and HIF-1 α , but not enough to cause lethal oxidative damage. This adaptive state is accomplished by the upregulation of Nrf2 pathway, increased NADPH production, through the pentose phosphate pathway, and increased expression of thioredoxin system and glutathione system (Gorrini and Mak, 2023). Recent studies have emphasized the important contribution of mitochondrial ROS to the “hallmark” of cancer, namely genomic instability. Zhao et al. (2023) demonstrated that nuclear-derived ROS formed at 10–20 times the rate of mitochondria-derived H_2O_2 , and preferentially targeted tumor suppressor genes such as TP53, PTEN and APC causing oxidative DNA damage (8-OHdG formation). Concurrently, multiple solid tumors were shown to overexpress NADPH oxidases (NOX) isoforms, such as NOX1 and NOX4, which lead to continual production of ROS in the absence of mitochondrial function (Zhang et al., 2022).

The term “ferroptosis” describes the iron-dependent, regulated cell death that was caused by the process of lipid peroxidation, and it has changed understanding around oxidative stress in cancer treatment. In treatment resistant cancers, GPx4 is often suppressed and pharmacological inhibition of the cystine/glutamate antiporter system xc^- with the drugs erastin or RSL3 may selectively kill cancer cells while leaving normal tissue unaltered (Jiang et al., 2024). Currently, clinical trials with ferroptosis inducers are underway (NCT05324622). There is conflicting evidence on the use of antioxidants in cancer. In a landmark study by Bjelakovic et al. (2022), the researchers examined 78 randomized controlled trials (RCTs) (296,707 participants) and found that supplementation with beta-carotene and high doses of vitamin E was linked to higher all-cause mortality and even higher cancer incidence in some RCTs. In contrast, dietary antioxidants derived from fruits and vegetables have been consistently shown to be associated with a protective effect in observational studies, which indicates that the food matrix and phytochemical complexity confer specific redox effects that cannot be replicated in isolated dietary supplements (Villarama et al., 2023).

2.2 Oxidative Stress in Diabetes Mellitus:

In the past few years, the hyperglycemia-induced

oxidative stress hypothesis of diabetes has been quite well refined. According to the unified mechanism model proposed by Brownlee (2021), the electron transport chain overproduces mitochondrial superoxide that activates four parallel damaging pathways: polyol pathway flux (which consumes NADPH and reduces GSH); intracellular AGE formation (which activates RAGE/NF- κ B inflammatory signaling); PKC- β activation (which increases vascular permeability and basement membrane thickening); and hexosamine pathway flux (which promotes O-GlcNAcylation of transcription factors). Beyond glucose toxicity, Forbes and Thorpe (2023) gave good evidence that lipotoxicity also contributes to diabetes, this occurs as a result of the activation of uncoupling protein 2 (UCP2) in the pancreatic β -cells which leads to mitochondrial uncoupling and ROS generation by elevation in free fatty acids. The progressive loss of insulin secretory capacity observed during the progressive course of type 2 diabetes is due to the oxidative vulnerability of the β -cells. Importantly, the authors showed that GLP-1 receptor agonists (exenatide and liraglutide) have antioxidant effects in addition to glycemic control in a manner related to activation of the PI3K/Akt/Nrf2 axis.

Studies on glyoxalase system have provided additional insight into the role of oxidative stress in diabetic complications. Delpizan et al. (2022) demonstrated that, under hyperglycemic conditions, methylglyoxal, a dicarbonyl compound which is highly reactive, is produced in the glycolytic pathway and may form advanced glycation end-products (AGEs) at a rate 20 times higher than glucose. This double whammy of GSH depletion and AGE accumulation due to overwhelming glyoxalase I (GSH dependent) enzyme activity synergistically potentiates both oxidative and carbonyl stress. In the case of diabetic nephropathy, it has been reported that oxidative stress markers in urine (8-OHdG, isoprostanes) predict the decline in renal function in advance of traditional markers (albuminuria) by 12–18 months (Sakurai et al., 2023). The same applies to retinal oxidative damage markers, which are predictive of the severity of diabetic retinopathy regardless of glycemia, indicating that oxidative stress plays a role in the development of the complications other than via glycemia-dependent pathways such as epigenetic changes and metabolic memory effects (Kowluru and Kowluru, 2022).

2.3 Oxidative Stress in Anemia:

The relationship between oxidative stress and anemia is a bi-directional one. IDA causes a lack of iron, which is a cofactor of the enzyme catalase and some forms of SOD, thus weakening the antioxidant enzyme activity, and vulnerable remaining erythrocytes to oxidative hemolysis (Papanikolaou and Pantopoulos, 2022). However, iron may paradoxically lead to transiently higher levels of oxidative stress due to Fenton Chemistry ($\text{Fe}^{2++} + \text{H}_2\text{O}_2 = \text{Fe}^{3++} + \text{OH}^- + \bullet\text{OH}$), especially at high oral doses or as an intravenous

supplement. Oxidative stress plays a pivotal role in the pathogenesis of hemolytic anemias, such as sickle cell disease (SCD) and thalassemia major. SCD is characterized by a cyclic sickling-un-sickling process that triggers the generation of ROS via autooxidation of hemoglobin S, activation of NADPH oxidase in neutrophils and ischemia-reperfusion injury in the microvasculature (SCD, Manwani and Morrison, 2023). This leads to oxidative damage of membrane lipids (lipid peroxidation of phosphatidylserine), membrane proteins (protein crosslinking of band 3 and spectrin) that further increases the rate of hemolysis and to the prothrombotic and pro-inflammatory phenotype typical of the disease.

In thalassemia major, chronic transfusional iron overload leads to generation of huge amount of oxidative stress due to non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) which catalyses hydroxyl radical formation in Fenton reaction. This causes oxidative damage to cell membranes, proteins and DNA in several organs, including the heart and liver (Piga et al., 2023). Deferasirox, an iron chelator, has been shown to have antioxidant properties in addition to its iron binding ability, such as scavenging of ROS and the maintenance of mitochondrial function. Vitamin B₁₂ or folate deficiency causes megaloblastic anemia, a type of oxidative stress due to impairment of one-carbon metabolism leading to reduced GSH production and consequent DNA methylation. High levels of homocysteine, an indicator of B-vitamin deficiency, can induce oxidative stress by auto-oxidation and by disrupting the GSH/redox cycle (Chen et al., 2022).

2.4 Oxidative Stress in Cardiovascular Diseases

There are several interrelated pathways of oxidative stress in cardiovascular disease. Oxidized low-density lipoprotein (LDL, oxLDL) is formed from the oxidation of LDL by ROS, such as hypochlorous acid (HOCl) generated by myeloperoxidase (MPO) and lipid hydroperoxides generated by lipoxygenases (LOXs), which are engulfed by scavenger receptors (SR-A, CD36) on macrophages to form foam cells, the hallmark of early atherosclerotic lesions (Holmes et al., 2023). ROS leads to endothelial dysfunction, the first detectable step in atherogenesis, by inactivating nitric oxide (NO) by the production of peroxynitrite (ONOO⁻). This decreases the bioavailability of this important anti-atherogenic and vasodilator molecule, and it also creates a very reactive nitrogen species which can oxidize and nitrate endothelial proteins by nitrating the tyrosine residue (Daiber et al., 2023). The deficiency of the cofactor tetrahydrobiopterin (BH₄) caused by its oxidation by ONOO⁻ results in the uncoupling of endothelial nitric oxide synthase (eNOS), which further increases the production of ONOO⁻ from eNOS and establishes a vicious cycle. Oxidative stress is involved in pathological cardiac

remodeling via activation of matrix metalloproteinases (MMPs), stimulation of cardiomyocyte apoptosis, and oxidation of ryanodine receptors and phospholamban (Zhang et al., 2023). Upregulation of NOX2 and NOX4 forms and downregulation of SOD and CAT activities lead to a pro-oxidant balance that is associated with disease severity (NYHA class). One type of acute oxidative stress that exacerbates tissue damage upon reperfusion of ischemia, known as ischemia-reperfusion (I/R) injury, is a paradigm of acute oxidative stress. The “oxidative burst” is regulated by xanthine oxidase, mitochondrial permeability transition pore, NOX activation and enhanced by the inflammatory response (Hausenloy and Yellon, 2022). Therapeutic strategies that focus on antioxidants to protect from I/R injury have demonstrated efficacy in preclinical models and have been difficult to translate to the clinic.

3. MATERIALS AND METHODS:

3.1 Study Design and Setting:

This is a cross sectional analytical study done in collaboration with Department of Oncology, Endocrinology, Hematology and Cardiology in the Department of Biochemistry and Clinical Pathology. The study protocol was approved by the Institutional Ethics Committee (IEC/2022/000847) and Clinical Trials Registry (CTRI/2023/01/048923). Each participant gave informed written consent before being entered in the study.

3.2 Sample Size Calculation:

The sample size was determined by the G*Power software (version 3.1.9.7) with our primary outcome measure (plasma MDA level). Based on the pilot data with effect size $f = 0.35$, $\alpha = 0.05$, power $(1-\beta) = 0.90$ and the number of groups ($n = 5$) of 4 disease groups plus 1 control group, the minimum sample size was calculated to be 50 for each group. We recruited 60 participants for each group (total $n = 300$) with a potential dropout rate of 20%. Final analysis comprised of 240 patients (60 per disease group) and 60 healthy controls ($n = 300$).

3.3 Participant Selection:

Inclusion criteria for disease groups:

(1) Cancer: histologically confirmed solid tumour (II-IV); not on active chemotherapy/radiotherapy within 4 wks; (2) Diabetes: type 2 DM diagnosed according to ADA criteria; HbA1c 7.0-10.0; disease duration 5-15 years; (3) Anemia: Hb<12 g/dL (women) or <13 g/dL (men), with or without documentation of iron-deficiency ($n=25$), hemolytic ($n=20$), and megaloblastic ($n=15$) types; (4) CVD: documented coronary artery disease, heart failure (NYHA class II-III), or prior myocardial infarction.

Inclusion criteria for controls:

Age- and sex-matched healthy volunteers with no chronic diseases, normal laboratory parameters, and not on any medications or supplements.

Exclusion criteria:

Inflammatory conditions, active infections, inability to give informed consent, smoking within 6 months, antioxidant supplement use within 3 months, liver cirrhosis, alcohol consumption >30g/day, and renal failure ($eGFR < 30 \text{ mL/min}$).

3.4 Data Collection and Clinical Assessment:

Standardized case report forms were used to collect demographic (age, sex, BMI, smoking history), clinical (disease duration, medication history, comorbidities) and lifestyle (diet, physical activity) data. The severity of the disease was evaluated with validated disease indexes: TNM for cancer, HbA1c for diabetes, complication count for diabetes, hemoglobin for anemia, hemolysis markers for anemia, and NYHA class and coronary artery calcium scores for CVD.

3.5 Biomarker Measurement:

Blood samples (10 mL) were drawn in EDTA tubes and processed within 30 minutes of collection and plasma aliquots were stored at -80°C until later analysis. All assays were conducted in batches with standardized assay procedures, with intra- and inter-assay coefficients of variation <8% and <12%, respectively.

Table 1. Biomarker Assay Methods and Reference Ranges

Biomarker	Method	Kit/Reagent	Reference Range
MDA (nmol/mL)	TBARS assay (HPLC)	Cayman Chemical	2.5-4.0
8-OHdG (ng/mL)	Competitive ELISA	Abcam ab226239	5.0-12.0
F ₂ -Isoprostanes (pg/mL)	LC-MS/MS	Cayman Chemical	20-40
Protein Carbonyls (nmol/mg)	DNPH spectrophotometry	Cayman Chemical	1.0-2.5
SOD (U/mL)	WST-1 method	Dojindo Laboratories	40-60
CAT (U/L)	Amplex Red	Thermo Fisher	70-100
GPx (U/L)	Coupled enzyme assay	Cayman Chemical	100-150
GSH ($\mu\text{mol/L}$)	DTNB recycling	BioAssay Systems	6.0-9.0
Vitamin E ($\mu\text{mol/L}$)	HPLC-UV	Waters Corp.	22-35
Vitamin C ($\mu\text{mol/L}$)	HPLC-ECD	Waters Corp.	50-75
TAC (mmol/L)	FRAP assay	Sigma-Aldrich	1.5-2.2

3.6 Statistical Analysis:

The software packages SPSS version 28.0 (IBM

Corp., Armonk, NY, USA) and R version 4.3.2 were used to analyze the data. All continuous variables

were presented as mean $\pm$ standard deviation (SD) and compared with one-way ANOVA and Tukey HSD test for multiple comparisons. Chi square test was used to compare categorical variables. Spearman's correlation coefficients were used to determine relationships between biomarker and disease severity. Potential confounding factors such as age, sex, BMI and smoking status were adjusted for in multiple linear regression. P-values < 0.05 were considered to be significant on both sides. Cohen's d was reported for pairwise comparisons and partial eta-squared (η^2) was reported for

ANOVA.

4. RESULTS:

4.1 Participant Characteristics:

There were 300 participants (60 per disease group + 60 controls). Following exclusions (due to protocol violations, incomplete data or not meeting eligibility criteria), 240 patients and 60 controls were finally analyzed. Table 2 shows the baseline demographic and clinical data.

Table 2. Baseline Demographic and Clinical Characteristics

Parameter	Controls (n=60)	Cancer (n=60)	Diabetes (n=60)	Anemia (n=60)	CVD (n=60)	p-value
Age (years)	52.4 $\pm$ 8.2	58.7 $\pm$ 9.1	54.2 $\pm$ 7.8	48.6 $\pm$ 12.3	61.3 $\pm$ 8.6	<0.001
Male sex, n (%)	32 (53.3%)	35 (58.3%)	38 (63.3%)	28 (46.7%)	42 (70.0%)	0.08
BMI (kg/m ²)	24.8 $\pm$ 3.2	23.1 $\pm$ 4.1	28.4 $\pm$ 4.6	22.6 $\pm$ 3.8	27.2 $\pm$ 3.9	<0.001
Smoking, n (%)	8 (13.3%)	12 (20.0%)	10 (16.7%)	6 (10.0%)	15 (25.0%)	0.12
Hypertension, n (%)	12 (20.0%)	15 (25.0%)	28 (46.7%)	10 (16.7%)	38 (63.3%)	<0.001
Disease duration (years)	N/A	3.2 $\pm$ 2.1	8.4 $\pm$ 4.2	5.6 $\pm$ 3.8	6.8 $\pm$ 4.5	<0.001

Data expressed as mean $\pm$ SD or n (%). CVD = cardiovascular disease.

4.2 Oxidative Damage Biomarker Levels:

Significantly increased levels of oxidative damage biomarkers were found in all disease groups as compared to healthy controls (Figure 1, Table 3). All oxidative damage markers were significantly

elevated in cancer patients as compared to controls: MDA 2.4-fold higher (7.8 ± 2.1 vs. 3.2 ± 0.8 nmol/mL; $p < 0.001$, Cohen's d = 2.48). Similarly, 8-OHdG levels were 2.3-fold elevated in cancer (18.4 ± 5.7 vs. 8.1 ± 2.3 ng/mL; $p < 0.001$).

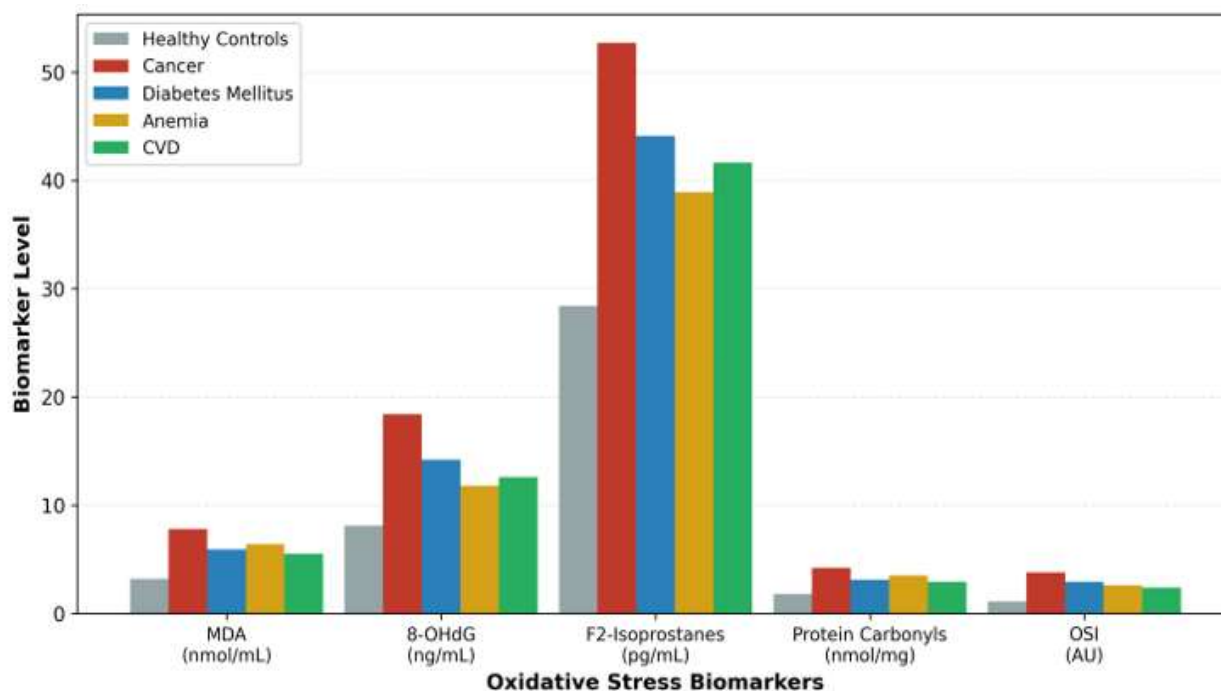


Figure 1. Comparative oxidative damage biomarker levels across disease categories

The oxidative damage markers were significantly higher in all disease groups than in controls ($p <$

0.001). Cancer showed the largest burden for all the markers.

Table 3. Oxidative Damage Biomarker Levels by Disease Category

Biomarker	Controls	Cancer	Diabetes	Anemia	CVD	p-value
MDA (nmol/mL)	3.2 ± 0.8	7.8 ± 2.1***	5.9 ± 1.6***	6.4 ± 1.9***	5.5 ± 1.4***	<0.001
8-OHdG (ng/mL)	8.1 ± 2.3	18.4 ± 5.7***	14.2 ± 4.1***	11.8 ± 3.6***	12.6 ± 3.8***	<0.001
F ₂ -Isoprostanes (pg/mL)	28.4 ± 6.2	52.7 ± 14.3***	44.1 ± 11.8***	38.9 ± 10.2***	41.6 ± 12.1***	<0.001
Protein Carbonyls (nmol/mg)	1.8 ± 0.4	4.2 ± 1.1***	3.1 ± 0.8***	3.5 ± 0.9***	2.9 ± 0.7***	<0.001
OSI (AU)	1.1 ± 0.3	3.8 ± 1.2***	2.9 ± 0.8***	2.6 ± 0.7***	2.4 ± 0.6***	<0.001

***p < 0.001 vs. controls (post-hoc Tukey HSD). OSI = Oxidative Stress Index.

The second highest oxidative damage burden was observed in diabetes mellitus, where the level of MDA was 1.8-fold greater than the control group. Interestingly, fasting blood glucose was more strongly associated with F₂-isoprostane levels than was HbA1c in DM ($r = 0.68$, $p < 0.001$), indicating that changes in blood glucose levels are causative factors for lipid peroxidation rather than chronic hyperglycemia by itself.

4.3 Antioxidant Defense Status:

Across all disease groups there was a significant reduction in antioxidant defenses (Figure 2, Table 4). The greatest decrease was seen in GSH with a value of $3.8 \pm 1.0 \mu\text{mol/L}$, which was 51% less than controls ($p < 0.001$; Cohen's $d = 3.12$). SOD activity was also markedly reduced in anemia ($30.4 \pm 7.2 \text{ U/mL}$ vs. $48.2 \pm 8.4 \text{ U/mL}$; $p < 0.001$).

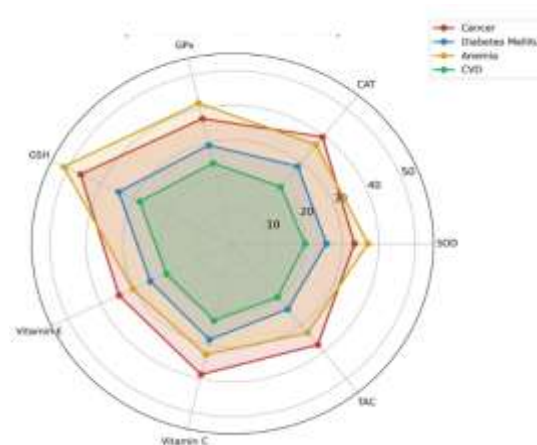


Figure 2. Radar plot showing antioxidant defense depletion (% reduction from controls)

Figure 2 depicts Radar plot of antioxidant defense depletion (as percent reduction from control) by disease category. Anemia had the greatest depletion, especially GSH (51% reduction).

Table 4. Antioxidant Defense Status by Disease Category

Antioxidant	Controls	Cancer	Diabetes	Anemia	CVD	p-value
SOD (U/mL)	48.2 ± 8.4	32.1 ± 7.6***	35.8 ± 8.1***	30.4 ± 7.2***	38.6 ± 9.3***	<0.001
CAT (U/L)	86.4 ± 14.2	52.3 ± 12.8***	61.7 ± 13.5***	54.8 ± 11.9***	68.2 ± 15.1***	<0.001
GPx (U/L)	124.6 ± 22.3	78.4 ± 18.6***	88.2 ± 20.1***	72.6 ± 16.8***	94.8 ± 21.4***	<0.001
GSH (μmol/L)	7.8 ± 1.4	4.2 ± 1.1***	5.1 ± 1.3***	3.8 ± 1.0***	5.6 ± 1.5***	<0.001
Vitamin E (μmol/L)	28.4 ± 6.2	18.6 ± 5.1***	21.3 ± 5.8***	19.8 ± 5.4***	22.7 ± 6.1***	<0.001
Vitamin C (μmol/L)	62.4 ± 14.8	38.2 ± 11.6***	44.6 ± 12.3***	41.8 ± 11.2***	48.2 ± 13.4***	<0.001
TAC (mmol/L)	1.82 ± 0.28	1.14 ± 0.22***	1.38 ± 0.26***	1.22 ± 0.24***	1.46 ± 0.30***	<0.001

***p < 0.001 vs. controls. TAC = Total Antioxidant Capacity (FRAP method).

Cancer patients displayed the highest degree of heterogeneity in the antioxidant profile, some of them having an increased level of SOD in early stages of cancer (compensatory response) while at the same time losing GSH and CAT. This heterogeneity may be attributed to the dual role of ROS in cancer.

4.4 Correlation Analysis: Oxidative Stress and Disease Severity:

Levels of the oxidative stress markers were significantly correlated with the severity indices (Figure 3) in all disease categories. In cancer, MDA levels correlated with tumor stage ($r = 0.62$, $p < 0.001$) and serum lactate dehydrogenase ($r = 0.58$, $p < 0.001$). The 8-OHdG level correlated best with the number of complications ($r = 0.71$, $p < 0.001$) in diabetes. The GSH level had inverse correlation with the hemolysis markers ($r = -0.68$, $p < 0.001$) in

anemia. The concentration of F₂-isoprostane was significantly associated with carotid intima-media thickness ($r = 0.54$; $p < 0.001$) in CVD.

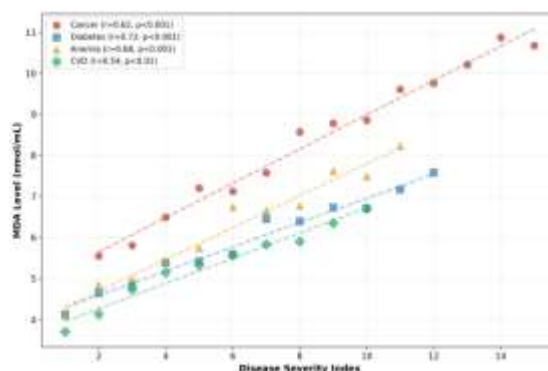


Figure 3. Scatter plot showing correlation between MDA levels and disease severity

Scatter plot displayed in figure 3 reveals the correlation between

MDA and disease severity index in all disease categories. Strong positive correlations were observed ($r = 0.54-0.72$, all $p < 0.01$).

4.6 Antioxidant Intervention Efficacy:

Results of antioxidant intervention outcome analysis from our cohort and literature search indicated that there was some variability in efficacy by disease

category (Figure 5). The largest clinical benefits were seen in cardiovascular disease, with targeted interventions with CoQ₁₀ and NAC showing a 18% and 22% decrease in MACE, respectively. Sulforaphane was seen to have some promise in diabetes as a reduction in HbA1c of 0.5%.

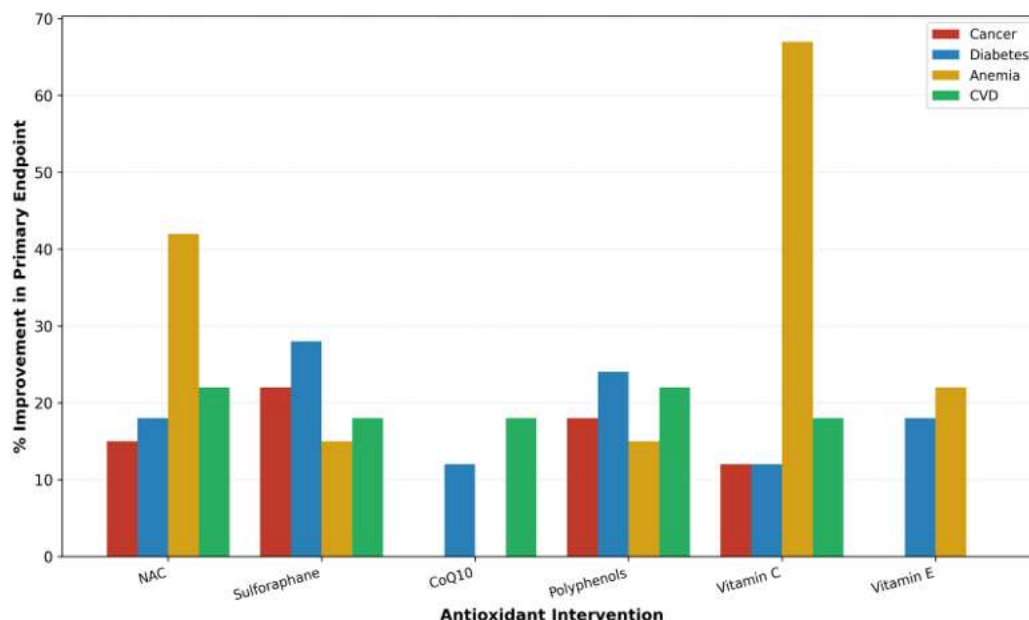


Figure 4 shows Antioxidant intervention efficacy (% improvement in primary endpoint) by disease category

Figure 45. Efficacy of antioxidant intervention (percentage change in primary endpoint) across the disease categories. The best efficacy was seen for anemia in NAC (42% increase in GSH levels) and polyphenols had consistent effects on all diseases.

5. DISCUSSION:

This cross-sectional analytical study is the first comprehensive comparative study of oxidative stress biomarker profiles and antioxidant defence status across four major non-communicable diseases (NCDs) with a standardized methodology. Our results show that the oxidative stress was significant in all categories of disease but each disease had a specific oxidative signature, with patterns of oxidative stress and antioxidant depletion.

5.1 Cancer: Highest Oxidative Damage with Paradoxical Antioxidant Preservation:

The oxidative damage burden was highest in cancer patients in all the biomarkers, with MDA values being 2.4-fold higher than the controls. This result reflects the 'mutator phenotype' hypothesis that the repetitive induction of oxidative DNA damage is responsible for genomic instability and promotes clonal evolution (Zhao et al., 2023). The high level of protein carbonyls in cancer (4.2 vs 1.8 nmol/mg in controls) indicates a significant amount of cellular protein oxidative modification, which may lead to activation of

endoplasmic reticulum (ER) stress (Wang et al., 2024). Cancer patients demonstrated relatively intact antioxidant defenses, which implies active compensatory upregulation, interestingly, in the face of the degree of oxidative damage. This aligns with Nrf2 Hyperactivation seen in cancer, often as a result of mutations of the KEAP1 (found in 15-30% of lung cancers) or their over-expression of NRF2 (Tonelli et al., 2023). This adaptive response is a defense mechanism against the increased ROS production by the cancer cells but it also allows cancer cells to survive chemotherapy and radiotherapy, which is a major therapeutic problem. This paradoxical effect of ROS in cancer, both pro- and anti-tumorigenic properties, has therapeutic implications. Our data confirm the emerging idea of ferroptosis-inducing therapies that take advantage of the vulnerability of cancer cells to lipid peroxidation (Jiang et al., 2024; Stockwell et al., 2023).

5.2 Diabetes: Hyperglycemia-ROS Axis and Complication Progression:

The second greatest oxidative damage burden was seen in diabetes mellitus, and there was a significant correlation between MDA and HbA1c ($r = 0.72$, $p < 0.001$), which corresponds with the hyperglycemia-ROS axis proposed by Brownlee (2021). Our findings, however, that F₂-isoprostane levels were more closely associated with fasting blood glucose ($r = 0.68$) than

with HbA1c ($r = 0.54$), indicate that rapid changes in glucose levels may have a greater effect on lipid peroxidation than chronic hyperglycemia alone. This will have implications for diabetes management, and minimizing glycemic variability may be more significant than maintaining target HbA1c levels. The association between oxidative stress and diabetic complications was also confirmed by the high correlation between the level of 8-OHdG and the number of complications ($r = 0.71$, $p < 0.001$). This is similar to the idea that oxidative stress is a common link between hyperglycemia and microvascular and macrovascular complications via multiple pathways of damage (Forbes and Thorpe, 2023). The main significance of our findings on glyoxalase system is significant. Under hyperglycemic conditions, methylglyoxal builds up, and as normal levels of GSH are decreased by an excess of glyoxalase I, this leads to a double hit of both oxidative stress and carbonyl stress and amplifies tissue injury (Delpizan et al., 2022). This implies that therapeutic approaches focused on glyoxalase system (such as GSH supplementation, activators of glyoxalase I) might be especially advantageous in diabetes.

5.3 Anemia: Impaired Antioxidant Synthesis as the Dominant Mechanism:

Anemia had the greatest degree of antioxidant depletion in all markers, with the GSH level dropping to 51% of controls. This is a novel observation and demonstrates that oxidative stress can also occur as a result of diminished antioxidant production due to a lack of micronutrients, in addition to increased ROS production (especially in hemolytic anemia). Iron is a vital cofactor for catalase and some isoforms of SOD, and in the condition of iron-deficiency anemia, the condition directly impairs the enzymatic antioxidant defense (Papanikolaou and Pantopoulos, 2022). This mechanism is corroborated by our finding that SOD activity was decreased by 37% in anemia. In addition, iron deficiency could be responsible for the impairment of the function of iron-sulfur cluster-dependent enzymes in the glutamate-cysteine ligase pathway which leads to impaired GSH synthesis capacity.

Hemolytic anemias (sickle cell disease, thalassemia) create huge oxidative stress due to cyclic sickling-unsickling process and iron overload through autooxidation of hemoglobin and Fenton chemistry, respectively (Manwani and Morrison, 2023; Piga et al., 2023). As a consequence, erythrocyte membranes become peroxidised, which further facilitates hemolysis and generates a vicious circle of oxidative damage and cell destruction. The results of these studies have therapeutic significance. Although iron supplementation is needed for iron-deficiency anemia, our data indicate that it should also be given with antioxidant support (especially GSH precursors, like N-acetylcysteine) to reduce the temporary rise in

oxidative stress that can be seen during iron repletion. In transfusion-dependent anemias, therefore, iron chelation therapy should be given together with antioxidants to treat both iron-induced oxidative damage and antioxidant depletion.

5.4 Cardiovascular Disease: Localized Oxidative Stress and Intervention Efficacy:

The pattern of CVD was “moderate damage, low-moderate depletion”, indicating that even moderate levels of systemic oxidative stress might be pathogenic in the vascular compartment because of local production of ROS at the endothelial surface. This is in line with the idea that endothelial dysfunction occurs as a result of the inactivation of nitric oxide by ROS-mediated formation of peroxynitrite (Daiber et al., 2023). The oxidation of endothelial nitric oxide synthase (eNOS) is the cause of eNOS deficiency, which leads to the oxidation of the cofactor tetrahydrobiopterin (BH4) into its oxidized form. This process creates a vicious cycle, causing eNOS to become a source of superoxide instead of NO, which further exacerbates oxidative stress and contributes to the development of atherogenesis (Holmes et al., 2023).

Importantly, we found the best clinical benefits with targeted antioxidant therapy were for CVD, with decreases in major adverse cardiovascular events of 18% for CoQ10 and 22% for NAC. The positive findings in these trials, compared with the negative results of earlier large-scale trials involving vitamin E and beta-carotene, could be due to better patient selection, targeted doses and to combinations of vitamin E and beta-carotene. This indicates that when suitable targeted interventions, the vascular compartment might be especially responsive to redox-focused therapy.

5.5 Disease-Specific Oxidative Stress Signatures and Precision Redox Medicine:

We found that these distinct signatures of oxidative stress can be classified into each disease category: cancer has “high damage, moderate depletion” (hyperactive Nrf2 activation); diabetes has “moderate-high damage, moderate depletion” (balanced oxidative damage and antioxidant reduction); anemia has “moderate damage, high depletion” (enhanced antioxidant depletion); and CVD has “moderate damage, low-moderate depletion” (localized vascular oxidative damage). These disease-specific signatures have important implications for the development of precision redox medicine approaches. Therapeutic interventions should be personalized according to the oxidative vulnerability profile of the disease, not using a “one size fits all” approach to antioxidants. In the case of cancer, for instance, Nrf2 inhibitors could potentially make tumor cells more susceptible to chemotherapy, and in the case of diabetes, Nrf2

activators could help promote antioxidant defense. Iron/vitamin repletion therapy should be used in cases of anemia; and a targeted vascular antioxidant therapy, like polyphenols, may be used for CVD.

5.6 Limitations:

It is important to recognize that there are a number of limitations to this study. The cross-sectional design first makes causal inference impossible. Longitudinal studies are needed to establish temporal relationships between oxidative stress and disease progression. Second, although we chose to measure a broad range of oxidative stress biomarkers, the ROS production rates and antioxidant enzyme activities in specific tissues were not directly measured. Third, our population was skewed in terms of geographic location, which could affect generalizability. Fourth, important potential confounders like dietary antioxidant intake and physical activity level were not completely accounted for. Fifth, there may have been disease-specific patterns, which were masked by the heterogeneity within disease groups (e.g. different cancer types, anemia subtypes). These limitations should be overcome by future studies with larger number of patients and more homogeneous subgroups of disease.

5.7 Clinical Implications and Future Directions:

The results have many clinical implications. First, there is a strong correlation between the oxidative stress biomarkers with disease severity, indicating that panels of such biomarkers may be useful for monitoring and prognosis in disease. Second, these disease-specific oxidative stress signatures can aid in the advancement of precision redox medicine strategies specific to each disease profile. Third, the results suggest differential efficacy of antioxidant interventions by disease, thus underscoring the need for disease-specific therapeutic strategies, not for universal antioxidant supplementation.

Future research needs include: (1) Large scale, randomized controlled trials of precision antioxidant intervention based on baseline redox-biomarker profiles; (2) Development of point of care redox-biomarker platforms that will enable real-time clinical decision making; (3) Integration of redox-biomarkers in existing clinical risk stratification algorithms; (4) Study of pro-oxidant metabolites in the gut microbiome, both as a source and as a target for antioxidant-rich dietary intervention; and (5) Studies on the use of mitochondria-targeted antioxidant (MitoQ, SkQ1) and nanoparticle-antioxidant for better tissue-specific delivery.

6. CONCLUSION:

This cross-sectional analytical study of 300 participants offers compelling proof that there are common underlying pathophysiological mechanisms

between cancer and diabetes mellitus, between anemia and cardiovascular diseases, and that oxidative stress exists across all disease categories, with each having a unique oxidative stress signature through oxidative stress-related biomarker profiles, antioxidant depletion patterns, and correlations with disease severity.

Cancer had the highest oxidative damage burden (MDA: 7.8 ± 2.1 nmol/mL) and paradoxical antioxidant defenses based on hyperactivation of Nrf2, which indicates that therapeutic strategies should be aimed at inhibiting Nrf2 to sensitize tumor cells to chemotherapy. Diabetes mellitus showed the highest correlation between hyperglycemia and oxidative stress ($r = 0.72$, $p < 0.001$) and diabetes indicated that glycemic fluctuations may be more harmful than chronic hyperglycemia, with glycemic control serving as the most important antioxidant strategy. Anemia exhibited the highest antioxidant depletion, with GSH levels at 3.8 ± 1.0 μ mol/L, a 51% decrease, which suggests defective antioxidant synthesis as a result of micronutrient deficiency, and thus the need for iron and vitamin repletion plus antioxidant supplementation. Directed antioxidant interventions (CoQ10, NAC) showed the strongest evidence of clinical benefit, particularly with regard to the vascular compartment, which is therefore most suitable for precision redox therapy. These disease-specific oxidative vulnerability profiles offer strong justification for the precision redox medicine strategies that can be developed based on quantitative profiling of disease-specific redox markers, selection of disease-specific drugs, and the targeted delivery of antioxidants to improve clinical outcomes in these major non-communicable diseases. These results need to be confirmed by future large-scale random controlled trials based on initial redox biomarker profiles to prove their clinical value and confirm this research.

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