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Evaluation of Oxidative Stress Biomarkers and Antioxidant Defences in Type 2 Diabetes: A Case-Control Analysis

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder characterized by chronic hyperglycaemia resulting from insulin resistance and progressive beta-cell dysfunction. Mounting evidence suggests that oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the capacity of the antioxidant defence system, plays a central role in the initiation, progression, and complications of T2DM. **Aim**: The present case-control study was designed to evaluate the levels of selected oxidant biomarkers and antioxidant defence enzymes in patients with T2DM and to compare them with age- and sex-matched healthy controls in a South Indian population. **Materials and Methods**: A hospital-based case-control investigation was conducted in the Department of Biochemistry, South India, between August 2011 and March 2012. Eighty (n=80) patients with T2DM diagnosed according to American Diabetes Association criteria and 80 age- and sex-matched apparently healthy controls were recruited. Serum malondialdehyde (MDA), protein carbonyls, 8-hydroxy-2'-deoxyguanosine (8-OHdG), advanced glycation end-products (AGEs), nitric oxide, total oxidant status (TOS), erythrocyte superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), reduced glutathione (GSH), vitamins C and E, and total antioxidant capacity (TAC) were estimated using validated spectrophotometric and ELISA techniques. Independent samples t-test, Pearson's correlation, and multivariate regression were used. **Results**: All oxidant biomarkers were significantly elevated in T2DM compared with controls ($p<0.001$), while all antioxidant biomarkers were significantly depleted ($p<0.001$). MDA correlated positively with HbA1c ($r=0.612$, $p<0.001$) and negatively with TAC ($r=-0.586$, $p<0.001$). **Conclusion**: T2DM is characterized by a profound pro-oxidant–antioxidant imbalance that correlates with glycaemic burden. Routine measurement of oxidative stress biomarkers may aid in risk stratification and in the design of antioxidant-supportive therapeutic strategies.

Keywords

Type 2 diabetes mellitus; Oxidative stress; Malondialdehyde; Antioxidant enzymes; Glutathione; HbA1c; Free radicals.

1. Introduction

Type 2 diabetes mellitus (T2DM) has emerged as one of the most prevalent metabolic disorders of the twenty-first century, affecting an estimated 537 million adults worldwide and projected to rise to 783 million by 2045 [1]. India alone is home to more than 100 million individuals with diabetes, earning it the unenviable distinction of the 'diabetes capital of the world' [2,3]. The chronic hyperglycaemia characteristic of T2DM is associated with multi-organ complications including diabetic retinopathy, nephropathy, neuropathy, and accelerated atherosclerosis [4].

Among the multiple pathogenic mechanisms underlying T2DM and its complications, oxidative stress occupies a pivotal position [5,6]. Reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical, are continuously generated as by-products of mitochondrial respiration, the polyol pathway, the hexosamine pathway, protein kinase C activation, and the formation of advanced glycation end-products (AGEs) [7,8]. Under physiological conditions, ROS are neutralised by enzymatic antioxidants — superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) — and non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin C, and vitamin E [9].

In T2DM, however, this balance is disrupted in two ways. First, chronic hyperglycaemia drives ROS overproduction through accelerated mitochondrial electron transport, glucose autoxidation, and the formation of AGEs that bind RAGE receptors and propagate further oxidative signalling [10]. Second, persistent oxidative load progressively depletes endogenous antioxidant defences, impairing their regenerative capacity [11]. The resulting net oxidative stress contributes directly to insulin resistance through serine phosphorylation of insulin receptor substrate-1, beta-cell dysfunction through pancreatic islet apoptosis, and microvascular complications through endothelial dysfunction [12,13].

Several oxidative stress biomarkers, each reflecting distinct molecular consequences of ROS attack, have been validated for clinical application. Malondialdehyde (MDA) is a classical marker of lipid peroxidation; protein carbonyls reflect oxidative protein damage; 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a sensitive marker of oxidative DNA injury; and total oxidant status

(TOS) and total antioxidant capacity (TAC) provide integrated measures of the redox milieu [14,15]. Although several studies have examined isolated biomarkers in Indian diabetics, comprehensive panels combining oxidant and antioxidant indices in well-defined South Indian cohorts remain sparse. The present study was therefore undertaken to characterise oxidative stress in T2DM patients and to evaluate its correlation with glycaemic control.

2. Materials and Methods

2.1 Study Setting

This hospital-based case-control study was conducted in the Department of Biochemistry, South India, between August 2011 and March 2012.

2.2 Participants

Eighty (n=80) consecutive patients with T2DM attending the Endocrinology Outpatient Department, diagnosed according to the American Diabetes Association criteria (fasting plasma glucose ≥126 mg/dL on two occasions, 2-hour post-prandial ≥200 mg/dL, or HbA1c ≥6.5%), were enrolled as cases [16]. Eighty age- and sex-matched apparently healthy individuals attending the master health check-up clinic served as controls. Inclusion criteria for cases comprised T2DM duration of ≥1 year and age 30–65 years. Exclusion criteria comprised type 1 diabetes mellitus, gestational diabetes, type 2 diabetes complicated by ketoacidosis, advanced renal or hepatic impairment, malignancy, smoking, alcohol use, regular antioxidant supplementation, and any acute infection within the preceding two weeks.

Table 1. Demographic and Baseline Clinical Characteristics

Variable	T2DM (n=80)	Controls (n=80)	p-value
Age (years)	52.4 ± 8.6	51.8 ± 8.2	0.642
Male, n (%)	46 (57.5)	44 (55.0)	0.749
BMI (kg/m²)	27.6 ± 3.4	24.2 ± 2.8	<0.001
Duration of T2DM (years)	6.4 ± 3.2	—	—
Fasting Plasma Glucose (mg/dL)	168.4 ± 38.6	92.4 ± 8.6	<0.001
HbA1c (%)	8.9 ± 1.6	5.4 ± 0.4	<0.001
Systolic BP (mmHg)	132.4 ± 12.4	121.6 ± 9.6	<0.001

2.3 Sample Collection

After overnight fasting, 10 mL of venous blood was collected by venipuncture into appropriate tubes — plain red-cap for serum biochemistry and oxidative stress markers, EDTA for whole-blood analyses (HbA1c, erythrocyte enzymes), and lithium heparin for plasma reduced glutathione. Samples were centrifuged at 3000 rpm for 10 minutes; serum/plasma was aliquoted and stored at –80°C until assay. Erythrocytes for SOD, catalase, and GPx assay were washed three times with cold normal saline and lysed before estimation.

2.4 Biochemical Estimations

Fasting plasma glucose was measured by the glucose oxidase–peroxidase method on an automated chemistry analyser (Beckman Coulter AU480). HbA1c was estimated using a Bio-Rad D-10 high-performance liquid chromatography system. Serum MDA was assayed by the thiobarbituric acid reactive substances method of Ohkawa et al. [17]. Protein carbonyls were quantified using the 2,4-dinitrophenylhydrazine spectrophotometric method [18]. 8-OHdG was assayed by competitive ELISA (Cell Biolabs, USA), and AGEs were estimated by autofluorescence-based spectrofluorimetry. Nitric oxide was estimated as nitrite using the Griess reagent method, and TOS was estimated using a commercial spectrophotometric kit (Rel Assay Diagnostics, Turkey).

Erythrocyte SOD activity was estimated by the pyrogallol autoxidation method of Marklund and Marklund [19]; catalase activity by the method of Aebi [20]; and GPx activity by the method of Rotruck et al. [21] using glutathione and t-butyl hydroperoxide as substrates. Reduced GSH was estimated using DTNB (5,5'-dithiobis-2-nitrobenzoic acid). Plasma vitamin C was measured by the dinitrophenylhydrazine method, plasma vitamin E by the colorimetric Emmerie–Engel method, and TAC using the FRAP (ferric reducing antioxidant power) assay [22].

2.5 Statistical Analysis

Data were analysed using IBM SPSS Statistics v26.0. Continuous variables were summarised as mean ± SD, and categorical variables as frequencies and percentages. Differences between cases and controls were assessed by independent samples t-test for continuous variables and chi-square test for categorical variables. Pearson's correlation coefficient was used to evaluate the relationships between glycaemic indices and oxidative stress biomarkers. Multivariate linear regression was performed with MDA as the dependent variable and HbA1c, BMI, age, and sex as predictors. A two-tailed p-value of less than 0.05 was considered significant.

3. Results

Demographic and baseline clinical characteristics are summarised in Table 1. Although the two groups were comparable in age and sex, T2DM patients had significantly higher BMI, fasting glucose, HbA1c, and systolic BP, in line with the expected metabolic phenotype.

All oxidant biomarkers were significantly elevated in the T2DM group compared with controls (Table 2). Serum MDA, the principal lipid peroxidation marker, was more than twofold higher in cases (4.86 vs 2.34 nmol/mL, $p<0.001$), confirming substantial lipid oxidative damage. Protein carbonyls, 8-OHdG, AGEs, nitric oxide, and TOS showed similar twofold increases. The pattern indicates oxidative damage across all major macromolecular substrates — lipids, proteins, and DNA — in T2DM.

Table 2. Oxidative Stress Markers in T2DM Patients and Controls

Oxidant Biomarker	**T2DM (Mean ± SD)**	**Controls (Mean ± SD)**	**p-value**
Malondialdehyde (MDA, nmol/mL)	4.86 ± 1.12	2.34 ± 0.62	<0.001
Protein Carbonyls (nmol/mg protein)	3.42 ± 0.94	1.68 ± 0.46	<0.001
8-OHdG (ng/mL)	18.6 ± 4.2	8.4 ± 2.1	<0.001
Advanced Glycation End-products (AU)	84.6 ± 18.4	46.2 ± 12.8	<0.001
Nitric Oxide (NO, µmol/L)	48.6 ± 11.2	32.4 ± 8.6	<0.001
Total Oxidant Status (µmol H_2O_2 Eq/L)	28.4 ± 6.4	14.6 ± 3.8	<0.001

Conversely, all antioxidant indices were significantly depleted in T2DM (Table 3). Erythrocyte SOD, catalase, and GPx activities were reduced by 42–48%, suggesting impaired enzymatic dismutation and detoxification of ROS. Reduced glutathione, the principal non-enzymatic antioxidant, fell by approximately 44%, reflecting compromised thiol homeostasis. Plasma vitamins C and E were also significantly reduced, and total antioxidant capacity (TAC) was diminished by approximately 38%.

Table 3. Antioxidant Defence Indices in T2DM Patients and Controls

Antioxidant Biomarker	**T2DM (Mean ± SD)**	**Controls (Mean ± SD)**	**p-value**

Superoxide Dismutase (U/mg Hb)	2.84 ± 0.62	4.92 ± 0.81	<0.001
Catalase (U/mg Hb)	48.6 ± 11.4	82.4 ± 14.6	<0.001
Glutathione Peroxidase (U/g Hb)	28.6 ± 6.8	46.4 ± 8.2	<0.001
Reduced Glutathione (mg/dL)	18.4 ± 4.6	32.8 ± 6.4	<0.001
Vitamin C (mg/dL)	0.62 ± 0.18	1.04 ± 0.22	<0.001
Vitamin E (mg/dL)	0.84 ± 0.21	1.32 ± 0.26	<0.001
Total Antioxidant Capacity (mmol/L)	1.16 ± 0.24	1.86 ± 0.32	<0.001

Pearson correlation analysis revealed strong positive associations of HbA1c with MDA ($r=0.612$, $p<0.001$), 8-OHdG ($r=0.548$, $p<0.001$), and AGEs ($r=0.586$, $p<0.001$), and strong negative associations with SOD ($r=-0.534$, $p<0.001$), GSH ($r=-0.498$, $p<0.001$), and TAC ($r=-0.586$, $p<0.001$). Multivariate regression analysis confirmed that HbA1c was an independent predictor of serum MDA ($\beta=0.482$, $p<0.001$), even after adjustment for age, sex, and BMI.

4. Discussion

The principal finding of the present study is that adult Indian patients with T2DM exhibit a marked pro-oxidant–antioxidant imbalance, characterized by significant elevations in lipid peroxidation, protein oxidation, DNA oxidation, and AGE accumulation, accompanied by significant depletion of enzymatic and non-enzymatic antioxidants. The robust correlation of these biomarkers with HbA1c reinforces the concept that chronic hyperglycaemia drives sustained oxidative damage.

Our finding of more than doubled serum MDA levels in T2DM patients aligns with the meta-analytic estimates of Picu et al. [23] and Bandeira et al. [24], both of whom reported significant elevations of lipid peroxidation markers in diabetic populations. The mechanistic basis of elevated MDA includes glucose autoxidation, mitochondrial superoxide leakage from the electron transport chain, and AGE-induced RAGE activation, all of which propagate hydroxyl radical formation and polyunsaturated fatty acid attack [25].

Protein carbonylation reflects irreversible oxidative damage to amino acid side chains, particularly lysine, arginine, proline, and threonine residues, and impairs the function of structural proteins, enzymes, and signalling intermediates [18]. The doubling of protein carbonyls observed in our T2DM patients suggests systemic protein oxidation — a pathway implicated in advanced diabetic complications including nephropathy and cataract formation. Similarly, the elevated 8-OHdG

levels reflect oxidative attack on guanine bases of nuclear and mitochondrial DNA, a marker linked with cancer risk and accelerated cellular ageing in chronic diabetes [26].

The reduction in SOD, catalase, and GPx activities in our T2DM cohort is consistent with the findings of Maritim et al. [11] and Sayyed et al. [27]. Several mechanisms underlie this depletion: glycation of antioxidant enzymes inactivates them, sustained oxidative load accelerates their consumption, and impaired transcription of antioxidant response element-driven genes (Nrf2 pathway) reduces de novo synthesis [28]. The substantial reduction in reduced glutathione further indicates compromised thiol redox balance and reduced regenerative capacity of GSH from oxidised glutathione (GSSG).

Our observation of reduced plasma vitamins C and E in T2DM concurs with the multicentre observations of Ceriello [12] and Singh et al. [29]. Both vitamins serve as critical chain-breaking antioxidants and their depletion accelerates lipid and protein oxidation. The integrated marker — total antioxidant capacity — captures the cumulative deficit, with our patients showing a 38% reduction. This deficit lays the biochemical foundation for the development and progression of microvascular and macrovascular complications [30].

The strong correlation of MDA, 8-OHdG, and AGEs with HbA1c, demonstrated in our regression analysis, has direct clinical implications. It identifies glycaemic control as the most important modifiable determinant of oxidative stress and therefore reinforces tight glycaemic control as a foundational therapeutic objective. Beyond this, several adjunctive strategies aimed at antioxidant restoration — including alpha-lipoic acid, N-acetylcysteine, vitamin E with caution, and Mediterranean-type dietary patterns — have demonstrated efficacy in modulating oxidative stress, although results in clinical end-point trials remain mixed [31].

The strengths of the present study include its case-control design, comprehensive biomarker panel covering oxidant and antioxidant arms, controlled exclusion criteria, and use of validated assays. Limitations include the cross-sectional nature, single-centre recruitment, lack of stratification by complication status, and absence of newer redox indicators such as oxidised glutathione (GSSG) and the GSH/GSSG ratio. Future longitudinal investigations should examine whether dynamic changes in oxidative biomarkers predict the development of microvascular complications and clarify the impact of antioxidant-rich dietary interventions in Indian diabetic populations.

5. Conclusion

Type 2 diabetes mellitus is associated with a marked pro-oxidant–antioxidant imbalance characterised by elevated lipid, protein, and DNA oxidation markers and depleted enzymatic and non-enzymatic antioxidants. These oxidative perturbations correlate strongly with glycaemic control as reflected by HbA1c. The findings reinforce the central role of oxidative stress in the pathogenesis of T2DM and support the integration of redox biomarker assessment into the clinical evaluation of diabetic patients. Therapeutic strategies aimed at restoring redox balance, in conjunction with optimal glycaemic control, may offer additional benefits in preventing the long-term complications of diabetes.

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