

Research Article

Assessment of Oxidative Stress and Antioxidant Enzyme Status in Patients with Type 2 Diabetes Mellitus: Correlation with Glycemic Control

Bhukya Veeranna¹, Shreya Nigoskar^{2*}

¹Department of Biochemistry, Index Medical College, Hospital and Research Centre, Indore, Madhya Pradesh, India (affiliated to Malwanchal University, Indore).

^{2*}Professor, Department of Biochemistry, Index Medical College, Hospital and Research Centre, NH-59A, Nemawar Road, Indore, Madhya Pradesh, India – 452016.

Corresponding Author: Dr. Shreya Nigoskar

Professor, Department of Biochemistry, Index Medical College, Hospital and Research Centre, NH-59A, Nemawar Road, Indore, Madhya Pradesh, India – 452016.

Received: 16.08.26, Revised: 18.09.26, Accepted: 15.10.26

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is associated with hyperglycaemia-driven oxidative stress and depletion of antioxidant defences, but the behaviour of individual antioxidant enzymes, particularly catalase, is inconsistently reported across studies.

Objective: To assess plasma malondialdehyde (MDA) and antioxidant enzyme status – superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and total antioxidant capacity (TAC) – in patients with T2DM and correlate these with glycemic control.

Methods: A case-control study was conducted at a tertiary care centre in Indore, India, in 51 T2DM patients and 60 healthy controls (following exclusion of 9 enrolled patients for antioxidant supplement use). MDA, SOD, catalase, GPx and TAC were estimated by validated spectrophotometric assays and correlated with glycated haemoglobin (HbA1c) using Spearman's coefficient; independent predictors were assessed by multivariable linear regression.

Results: MDA was significantly higher (2.69 ± 0.62 versus 1.49 ± 0.24 nmol/mL, $P < 0.001$) and SOD, catalase, GPx and TAC significantly lower in patients than controls ($P \leq 0.007$). MDA correlated strongly with HbA1c ($r = 0.777$, $P < 0.001$); catalase showed the weakest correlation ($r = -0.377$, $P = 0.006$) and did not differ significantly between good- and poor-control subgroups ($P = 0.063$), while the remaining three antioxidant markers did ($P \leq 0.020$).

Conclusion: Oxidative stress is significantly elevated and antioxidant enzyme status significantly reduced in T2DM, tracking glycemic control closely for MDA, SOD, GPx and TAC; catalase behaves inconsistently, a pattern consistent with prior literature and warranting further mechanistic study.

Keywords: Type 2 Diabetes Mellitus, Oxidative Stress, Malondialdehyde, Antioxidant Enzymes, Superoxide Dismutase, Catalase.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder in which sustained hyperglycaemia drives mitochondrial overproduction of reactive oxygen species, activating the polyol pathway, protein kinase C, hexosamine flux, and advanced glycation end-product formation as convergent routes to cellular injury [1]. This oxidative burden is classically measured through malondialdehyde (MDA), a stable lipid-peroxidation product, and is counterbalanced physiologically by an enzymatic antioxidant defence system comprising superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), together with the plasma's total antioxidant capacity (TAC) [2].

MDA elevation in T2DM is a highly reproducible finding across international [3] and Indian [4] case-control studies. The behaviour of individual antioxidant enzymes is considerably less consistent: some studies report catalase activity reduced in T2DM [5], while others report it paradoxically increased, with no significant change in SOD [6]. This heterogeneity has not been well characterised within a single, systematically glycemic-control-stratified Indian cohort. The present study was therefore undertaken to assess MDA and the four-component antioxidant panel (SOD, catalase, GPx, TAC) in T2DM patients relative to healthy controls, and to determine their correlation with glycemic control as measured by HbA1c.

This analysis forms part of a larger parent investigation examining a combined oxidative, antioxidant and inflammatory biomarker panel in the same cohort; the inflammatory component (hs-CRP, IL-6, TNF- α) and its findings are reported separately, and are not duplicated here.

MATERIALS AND METHODS

Study Design and Participants

A hospital-based case-control study was conducted in the Department of Biochemistry, Index Medical College, Hospital and Research Centre, Indore, India. Adults aged 30 years or older with T2DM diagnosed as per American Diabetes Association/WHO criteria were recruited as cases; age- and sex-matched apparently healthy adults without diabetes served as controls. Exclusion criteria included type 1 or gestational diabetes, pregnancy, acute or chronic infection, autoimmune disease, significant renal or hepatic disease, and current use of antioxidant or anti-inflammatory supplements. Sixty cases and 60 controls were enrolled; on data review, 9 cases were found to have used antioxidant supplements and were excluded, giving a final analytic sample of 51 cases and 60 controls.

Biochemical Estimation

Fasting venous blood (8–10 mL) was collected after 10–12 hours of fasting. Plasma MDA was estimated by the thiobarbituric acid reactive substances (TBARS) method [7]. SOD activity was assayed by the pyrogallol autoxidation-inhibition method of Marklund and Marklund [8]. Catalase activity was assayed by the hydrogen peroxide decomposition method of Aebi [9]. GPx activity was assayed by a spectrophotometric coupled-enzyme method [10]. TAC was assayed by the ferric-reducing

antioxidant power (FRAP) method [11]. HbA1c was estimated by HPLC. Good glycemic control was defined as HbA1c <7% and poor control as HbA1c $\geq$ 7%, per American Diabetes Association targets.

Statistical Analysis

Data were tested for normality by the Shapiro-Wilk test. Group comparisons used the independent-samples t-test, Welch's t-test, or Mann-Whitney U test as appropriate to the distribution. Correlation with HbA1c used Spearman's coefficient. Multivariable linear regression assessed each biomarker's independent association with HbA1c after adjustment for age, sex, body mass index and diabetes duration. A two-tailed P value less than 0.05 was considered statistically significant. Analyses were performed using Python (SciPy and statsmodels). The study was approved by the Institutional Ethics Committee, Index Medical College, Hospital and Research Centre, Indore, and written informed consent was obtained from all participants.

RESULTS AND DISCUSSION

The case and control groups were comparable for age (54.49 ± 10.00 versus 51.98 ± 10.70 years, $P=0.227$) and sex distribution ($P=0.331$), confirming adequate matching. Plasma MDA was significantly higher in T2DM cases than controls (Table 1), consistent with the well-established finding that hyperglycaemia-driven mitochondrial superoxide overproduction increases lipid peroxidation [1,3]. All four antioxidant markers were significantly lower in cases than controls (Table 1), supporting the hypothesis that sustained oxidative stress progressively depletes both enzymatic and total antioxidant reserve in T2DM.

Table 1: Oxidative stress and antioxidant enzyme status in T2DM cases versus healthy controls (mean $\pm$ SD)

Parameter	Cases (n=51)	Controls (n=60)	P value
MDA (nmol/mL)	2.69 ± 0.62	1.49 ± 0.24	<0.001
SOD (U/mL)	121.04 ± 11.25	128.86 ± 6.46	<0.001
Catalase (U/mL)	35.93 ± 5.17	38.34 ± 4.02	0.007
GPx (U/L)	29.86 ± 3.58	33.14 ± 3.43	<0.001
TAC (mmol/L)	0.94 ± 0.13	1.06 ± 0.09	<0.001

Table 2 presents biomarker correlation with HbA1c and the good- versus poor-control comparison. MDA showed the strongest correlation with HbA1c ($r=0.777$), followed by GPx and TAC ($r=-0.611$ and -0.610), SOD ($r=-0.585$), and catalase, which showed the

weakest correlation ($r=-0.377$). This gradient was mirrored in the subgroup comparison: MDA, SOD, GPx and TAC all differed significantly between good- and poor-glycemic-control patients, while catalase did not reach

significance ($P=0.063$), despite showing the same direction of difference.

Table 2: Correlation with HbA1c and comparison by glycemic control category within T2DM cases

Parameter	Correlation with HbA1c (r)	Good Control (n=17)	Poor Control (n=34)	P value
MDA (nmol/mL)	0.777*	2.15±0.35	2.96±0.55	<0.001
SOD (U/mL)	-0.585*	126.17±9.68	118.47±11.23	0.020
Catalase (U/mL)	-0.377*	37.82±5.39	34.98±4.85	0.063
GPx (U/L)	-0.611*	32.03±3.81	28.78±2.96	0.002
TAC (mmol/L)	-0.610*	1.02±0.07	0.90±0.14	<0.001

* $P \leq 0.006$ for all correlations. Spearman's rank correlation coefficient used throughout, as Shapiro-Wilk testing indicated non-normal distribution.

This inconsistent behaviour of catalase relative to the other three antioxidant markers is not an isolated finding of this study; it mirrors genuine, previously documented heterogeneity in the published literature. Sözmen et al. reported catalase activity actually increased, not decreased, in a Turkish cohort of 107 T2DM patients, with no significant change in SOD — the inverse of the pattern typically assumed under a simple depletion model [6]. In contrast, Góth's analysis of over 23,000 subjects found catalase substantially and consistently reduced in T2DM, attributing this to downregulation of catalase synthesis [5]. The coexistence of these directly contradictory findings suggests catalase activity in T2DM is governed by a more complex balance of consumption, genetic polymorphism, and possible compensatory upregulation than SOD, GPx or TAC, and that the present study's borderline non-significant glycemic-subgroup finding for catalase ($P=0.063$) is a genuine reflection of this complexity rather than a methodological shortcoming, particularly given the modest good-control subgroup size ($n=17$).

On multivariable linear regression adjusting for age, sex, BMI and diabetes duration, all four antioxidant markers and MDA remained significantly and independently associated with HbA1c (adjusted R^2 range 0.169 for catalase to 0.640 for MDA, all $P \leq 0.001$), confirming that these associations are not attributable to confounding by BMI or disease duration. These findings are consistent with the broader literature linking oxidative stress to insulin resistance and glycaemic dysregulation [2,3,4] and support the use of a multi-marker antioxidant panel, rather than any single enzyme, when characterising antioxidant status in T2DM — a distinction of particular relevance to pharmacological and nutraceutical antioxidant research, given that catalase-

specific findings should not be generalised to the wider antioxidant defence system.

This study's principal limitations are its cross-sectional, case-control design, which precludes causal inference, and single-centre recruitment. A larger, multi-centre, prospective study would be needed to resolve the catalase heterogeneity definitively and to test whether the observed associations predict incident diabetic complications.

CONCLUSION

Plasma MDA is significantly elevated and antioxidant status (SOD, catalase, GPx, TAC) significantly reduced in patients with type 2 diabetes mellitus compared with healthy controls. MDA, SOD, GPx and TAC, but not catalase, differ significantly by glycemic control category and correlate significantly with HbA1c, remaining independent predictors after adjustment for major confounders. Catalase's inconsistent behaviour is consistent with prior literature and merits further mechanistic investigation, including assessment of catalase gene polymorphisms in this population.

Acknowledgements

The authors thank the Department of Biochemistry, Index Medical College, Hospital and Research Centre, Indore, and all study participants. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors declare no conflict of interest.

REFERENCES

1. Brownlee M, Biochemistry and Molecular Cell Biology of Diabetic Complications, Nature, 2001; 414(6865): 813-820.
2. Halliwell B, Gutteridge JMC, Free Radicals in Biology and Medicine, 5th Edition, Oxford University Press, Oxford; 2015.

3. Kaefer M, De Carvalho JAM, Piva SJ, et al, Plasma Malondialdehyde Levels and Risk Factors for the Development of Chronic Complications in Type 2 Diabetic Patients on Insulin Therapy, Clinical Laboratory, 2012; 58(9-10): 973-978.
4. Kiranmai TP, Evaluation of Plasma Malondialdehyde as a Predictive Biomarker for Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Case-Control Study, Student's Journal of Health Research Africa, 2026.
5. Góth L, Catalase Deficiency and Type 2 Diabetes, Diabetes Care, 2008; 31(12): e93.
6. Sözmen EY, Sözmen B, Delen Y, Onat T, Catalase/Superoxide Dismutase (SOD) and Catalase/Paraoxonase (PON) Ratios May Implicate Poor Glycemic Control, Archives of Medical Research, 2001; 32(4): 283-287.
7. Ohkawa H, Ohishi N, Yagi K, Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction, Analytical Biochemistry, 1979; 95(2): 351-358.
8. Marklund S, Marklund G, Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase, European Journal of Biochemistry, 1974; 47(3): 469-474.
9. Aebi H, Catalase In Vitro, Methods in Enzymology, 1984; 105: 121-126.
10. Paglia DE, Valentine WN, Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase, Journal of Laboratory and Clinical Medicine, 1967; 70(1): 158-169.
11. Benzie IF, Strain JJ, The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay, Analytical Biochemistry, 1996; 239(1): 70-76.