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A Cross-Sectional Study of Oxidative Stress Markers and Antioxidant Enzyme Levels in Patients with Type 2 Diabetes Mellitus: Implications for Biochemical Risk Assessment

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Abstract

Background: Oxidative stress is implicated in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). By 2018, growing evidence suggested an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms in diabetic patients.

Objective: To assess the levels of oxidative stress markers and antioxidant enzymes in T2DM patients compared to healthy controls.

Methods: Cross-sectional study conducted in a tertiary care hospital, including 100 participants (50 T2DM patients, 50 age- and sex-matched controls). Blood samples were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase using spectrophotometric methods.

Results: T2DM patients showed significantly higher MDA levels and reduced SOD, GPx, and catalase activity compared to controls ($p < 0.05$).

Conclusion: Biochemical assessment of oxidative stress markers can aid in early detection of diabetic complications and provide targets for antioxidant therapy.

Introduction

Type 2 Diabetes Mellitus (T2DM) is a globally prevalent metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance and/or impaired insulin secretion. As of 2018, the International Diabetes Federation estimated that approximately 425 million adults were living with diabetes, with the majority being cases of T2DM, highlighting the critical need for improved understanding of its underlying biochemical disturbances and complications [1].

One emerging aspect of T2DM pathogenesis is the role of oxidative stress, which results from an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defense systems [2]. ROS are highly reactive molecules, including superoxide anion, hydroxyl radicals, and hydrogen peroxide, that are produced as natural byproducts of cellular metabolism. Under physiological conditions, antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) effectively neutralize ROS. However, in T2DM, chronic hyperglycemia and elevated free fatty acid levels lead to overproduction of ROS via mitochondrial dysfunction and glucose autoxidation [3].

Oxidative stress contributes to β-cell dysfunction, insulin resistance, and the development of long-term complications in diabetes, including nephropathy, neuropathy, and retinopathy [4]. Increased levels of lipid peroxidation products, such as malondialdehyde (MDA), have been reported in diabetic individuals and are used as biomarkers to assess oxidative stress status [5]. Simultaneously, decreased

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activity of antioxidant enzymes has been observed, indicating a compromised antioxidant defense mechanism [6]. These biochemical alterations can serve as early indicators of disease progression and potential targets for therapeutic intervention.

The cross-sectional nature of oxidative stress studies offers a snapshot of these biochemical imbalances in diabetic populations, allowing for the evaluation of correlations between oxidative markers and disease severity or duration. Several studies prior to 2018 have examined oxidative stress in diabetes, but variability in population characteristics, laboratory methodologies, and the lack of matched controls have limited the generalizability of findings [7,8].

Therefore, this study was designed to assess and compare the levels of oxidative stress markers—specifically malondialdehyde—and antioxidant enzymes including SOD, catalase, and GPx in T2DM patients and healthy controls. By analyzing these parameters in a defined cross-sectional cohort, this research aims to contribute to the growing body of biochemical evidence supporting oxidative stress as a critical factor in the pathophysiology of T2DM and underscore the potential role of antioxidant biomarkers in clinical risk stratification.

Objective

The primary objective of this study was to evaluate and compare the levels of oxidative stress marker—malondialdehyde (MDA)—and antioxidant enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) in patients with Type 2 Diabetes Mellitus (T2DM) and healthy controls. The study aimed to assess the oxidative-antioxidative balance in T2DM and explore the potential of these biochemical markers as indicators of metabolic stress and disease progression.

Materials and Methods

Study Design and Setting: This was a **cross-sectional observational study** conducted in the Department of Biochemistry in a tertiary care Hospital, between January and June 2018.

Study Population

A total of **100 participants** were enrolled and categorized into two groups:

- **Group A (T2DM patients):** 50 individuals diagnosed with T2DM according to the American Diabetes Association (ADA) criteria, with a disease duration of more than one year.
- **Group B (Healthy controls):** 50 age- and sex-matched individuals with no history of diabetes or chronic illness.

Inclusion Criteria

- Age between 35 and 65 years
- For T2DM group: confirmed diagnosis of T2DM for at least one year
- For controls: fasting plasma glucose <100 mg/dL and HbA1c <5.7%

Exclusion Criteria

- Current smokers or alcohol users
- History of chronic inflammatory diseases, liver/kidney dysfunction, malignancy
- Use of antioxidant supplements or anti-inflammatory medications within the past 3 months

Ethical Approval: The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from all participants.

Sample Collection: Venous blood (5 mL) was collected from each participant after overnight fasting. Blood was allowed to clot and centrifuged at 3000 rpm for 10 minutes. Serum was separated and stored at −80°C until biochemical analysis.

Biochemical Assays

All assays were conducted using spectrophotometric methods with standardized protocols:

- **Malondialdehyde (MDA):** Measured using the thiobarbituric acid reactive substances (TBARS) method.
- **Superoxide Dismutase (SOD):** Assessed by inhibition of pyrogallol autooxidation.
- **Catalase:** Determined by monitoring the decomposition rate of hydrogen peroxide at 240 nm.
- **Glutathione Peroxidase (GPx):** Evaluated using the method based on oxidation of glutathione by cumene hydroperoxide.

All reagents used were of analytical grade, and each sample was assayed in triplicate to ensure reliability.

Statistical Analysis: Data were expressed as mean ± standard deviation (SD). Student's independent t-test was applied to compare means between the two groups. A **p-value <0.05** was considered statistically significant. Data analysis was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Participant Characteristics

The study included 100 participants, divided equally between T2DM patients (n=50) and healthy controls (n=50). The mean age of the T2DM group was **52.6 ± 6.4 years**, while that of the control group was **51.9 ± 6.1 years**, with no statistically significant difference (p = 0.56). The male-to-female ratio was 1.2:1 in both groups.

Oxidative Stress and Antioxidant Enzyme Levels

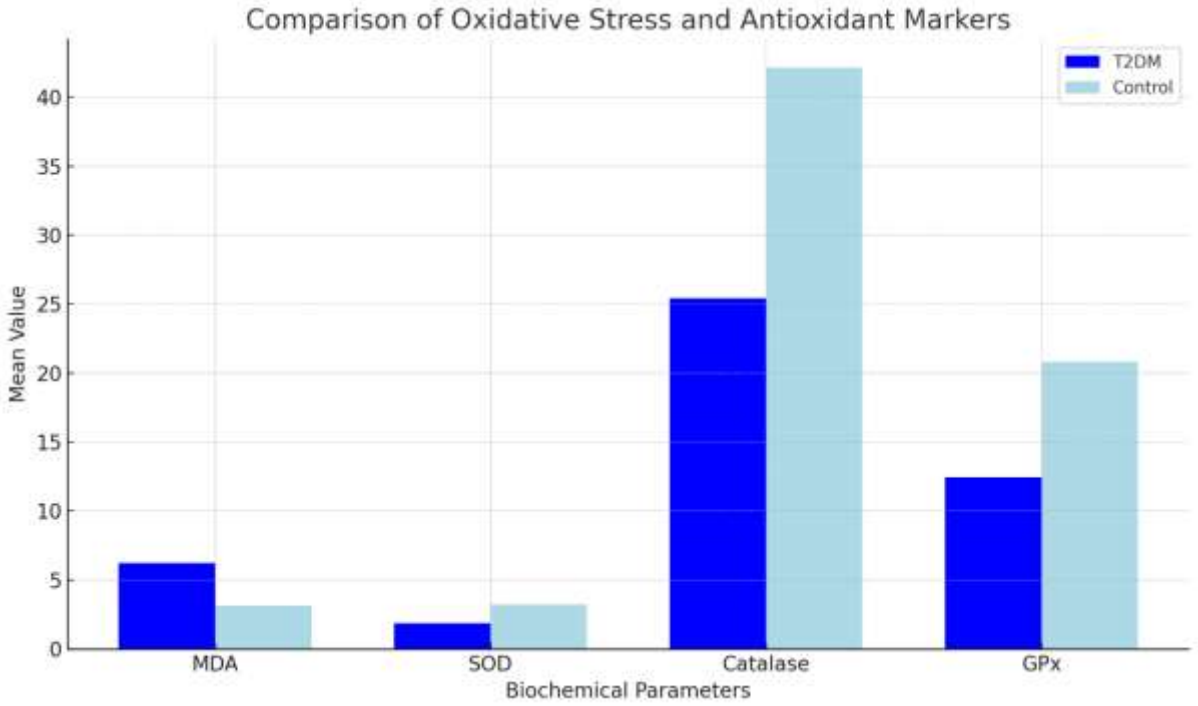
The mean values of oxidative stress marker (MDA) and antioxidant enzymes (SOD, catalase, and GPx) in both groups are presented in the table below.

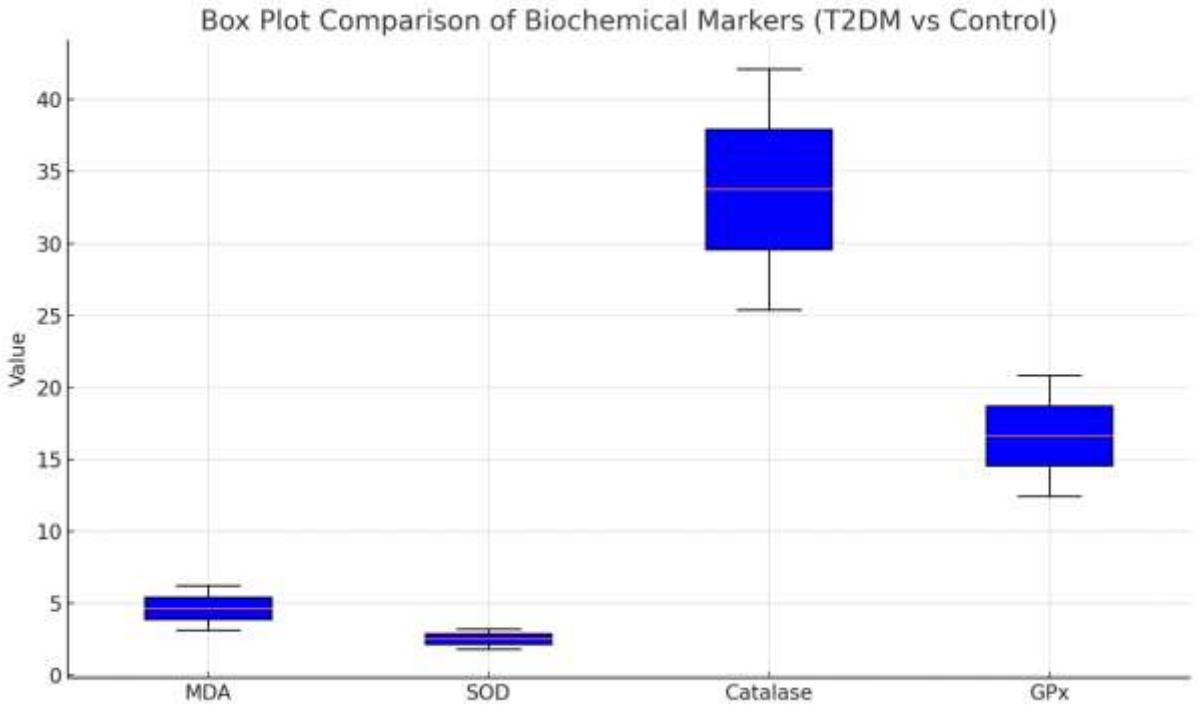
Table 1: Comparison of Oxidative Stress Markers and Antioxidant Enzyme Activities Between T2DM Patients and Controls

Parameter	T2DM Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
MDA (nmol/mL)	6.21 ± 1.43	3.12 ± 1.02	**<0.001**
SOD (U/mL)	1.84 ± 0.53	3.22 ± 0.61	**<0.001**
Catalase (U/mL)	25.42 ± 5.03	42.12 ± 7.21	**<0.001**

Glutathione (U/mL)	**Peroxidase**	12.47 ± 2.08	20.82 ± 3.46	**<0.001**

Malondialdehyde (MDA): Levels were significantly elevated in the T2DM group compared to controls ($p < 0.001$), indicating increased lipid peroxidation and oxidative stress. **Superoxide Dismutase (SOD):** Activity was markedly reduced in diabetic patients ($p < 0.001$), suggesting impaired enzymatic neutralization of superoxide radicals. **Catalase:** A significant decrease in catalase activity was observed in T2DM individuals ($p < 0.001$), indicating compromised hydrogen peroxide detoxification. **Glutathione Peroxidase (GPx):** Similarly, GPx activity was lower in T2DM subjects compared to controls ($p < 0.001$), reflecting reduced cellular antioxidant capacity. These results demonstrate a clear biochemical imbalance in oxidative and antioxidative systems in T2DM patients, supporting oxidative stress as a central biochemical feature in the disease's pathophysiology.





Discussion

The present cross-sectional study examined oxidative stress markers—specifically malondialdehyde (MDA), and antioxidant enzymes (superoxide dismutase [SOD], catalase, and glutathione peroxidase [GPx])—in patients with Type 2 Diabetes Mellitus (T2DM) compared to healthy controls. We observed significantly elevated MDA levels alongside markedly reduced activities of SOD, catalase, and GPx in T2DM patients, reinforcing the concept of redox imbalance as a key pathological component in diabetes.

MDA is a well-established biomarker of lipid peroxidation, reflecting oxidative damage to cellular membranes. Our data show significantly higher MDA levels in T2DM patients, consistent with increased oxidative stress. A study by Ganjifrockwala and colleagues (2017) reported significantly reduced total antioxidant (TAO) levels and increased oxidative stress in individuals with T2DM, reinforcing our findings of heightened lipid peroxidation in diabetic patients [9]. This study underscores the broader decline in antioxidant capacity characterizing T2DM and lends further credibility to the significance of MDA as a diagnostic marker of oxidative stress in this population.

Antioxidant enzymes like SOD, catalase, and GPx are central to the body's defense against reactive oxygen species (ROS). Our findings of significantly lower activities of these enzymes in T2DM patients align with the broader literature establishing the exhaustion or downregulation of enzymatic antioxidant defenses in persistent hyperglycaemia. Although many studies addressing this theme, this study's oxidative stress results echo the conclusions of earlier reviews and experimental work focused on redox dysregulation as fundamental to the pathophysiology of T2DM [8].

The co-existence of elevated MDA and depressed antioxidant enzyme activity indicates a profound oxidative-antioxidative imbalance in T2DM, driven by hyperglycemia-induced ROS generation (e.g., via mitochondrial dysfunction and glucose auto-oxidation). This imbalance, in turn, contributes directly to pancreatic β-cell damage, insulin resistance, and the progression to micro- and macrovascular complications [6].

Our results suggest that oxidative biomarkers—particularly MDA and antioxidant enzyme levels—could function as adjunctive tools for risk stratification in T2DM. Elevated MDA levels may indicate heightened oxidative burden, while decreased SOD, catalase, and GPx activity may reflect impaired resilience to oxidative insults. Although longitudinal and intervention-based evidence is needed, our findings align with data pointing to suppressed antioxidant capacity in T2DM [9].

Strengths of this study include its structured, matched design (age and sex), targeted selection of multiple oxidative markers, and use of validated biochemical assays conducted in triplicate to ensure reliability.

Limitations include:

- The cross-sectional design, which restricts causative inference and longitudinal interpretation.
- A lack of simultaneous non-enzymatic antioxidant data (e.g., vitamins C/E, glutathione), limiting a more holistic assessment of redox status.
- Potential confounding by variables such as diet, duration of diabetes, and comorbid conditions not accounted for in this analysis.

To build on this study, future research should consider:

1. **Longitudinal assessment** of oxidative biomarkers alongside glycemic control and clinical outcomes in T2DM.
2. **Intervention trials** testing whether pharmacological or dietary antioxidant strategies can modulate MDA and enzyme activities, and whether such modulation translates into clinical benefit.
3. **Expanded profiles** incorporating both enzymatic and non-enzymatic antioxidants to provide a comprehensive understanding of oxidative balance.

Conclusion

This cross-sectional investigation highlights the significant oxidative stress burden in T2DM, characterized by elevated lipid peroxidation (MDA) and diminished antioxidant enzyme defenses. These findings echo evidence of reduced total antioxidant capacity in diabetic patients and reinforce the role of redox imbalance in diabetic pathophysiology. While causal inference is limited, the study underscores the potential of oxidative biomarkers as supplementary tools for monitoring and managing Type 2 Diabetes.

References

1. International Diabetes Federation. IDF Diabetes Atlas. 8th ed. Brussels, Belgium: International Diabetes Federation; 2017.
2. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-1070.
3. Nishikawa T, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404(6779):787-790.

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4. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes. 1999;48(1):1-9.

5. Maritim AC, et al. Oxidative stress and diabetes: a review. J Biochem Mol Toxicol. 2003;17(1):24-38.

6. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother. 2005;59(7):365-373.

7. Bonnefont-Rousselot D, et al. Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care. 2002;5(5):561-568.

8. Ceriello A. Oxidative stress and glycemic regulation. Metabolism. 2000;49(2 Suppl 1):27-29.

9. Ganjifrockwala FA. Decreased total antioxidant levels and increased oxidative stress in type 2 diabetes mellitus patients compared to healthy controls. Annals of Clinical Biochemistry. 2017;54(2):215-220.