Indian J. Applied & Pure Bio. Vol. 40(1), 321-328 (2025). **ISSN: 0970-2091**
A Web of Science Journal

Estimation of oxidative stress in type 2 Diabetes mellitus patients in correlation with hbaic

S. Hari Priya[1*], Nagendra Babu[2], Praveen Kumar[3] and Triveni[4]

[1*,2,3,4]Department of Biochemistry, School of Allied Healthcare and Science, Malla Reddy University, Hyderabad - 500055 (India)

[1*]Corresponding author: Dr. S. Hari Priya

Mail ID: drharipriya@mallareddyuniversity.ac.in

Abstract

Diabetes mellitus (DM) is a multifactorial disorder that occurs when the body either does not produce enough insulin or cannot effectively use. Oxidative stress, which results from an imbalance between the production and elimination of free radicals, is a major contributor to poor glycemic control and complications in individuals with diabetes. To evaluate the level of oxidative stress in patients with type 2 diabetes mellitus (type 2DM) compared to healthy individuals. The study included a total of 120 participants, with 60 diagnosed with type 2 diabetes (test group) and 60 healthy individuals (control). Approval was obtained from the institutional ethical committee followed by venous blood sample was collected from diabetic and healthy subjects to measure fasting blood sugar (FBS mg/dl), postprandial blood sugar (PPBS mg/dl), HbA1c (%), total antioxidant capacity (TAC mmol/dl), and malondialdehyde (MDA mmol/dl). The data obtained was analyzed using SPSS software version 23.0. The results showed that diabetic patients had higher levels of HbA1c, FBS, PPBS, and MDA and lower levels of TAC compared to healthy subjects. The differences in these variables between the two groups were statistically significant. Patients with type 2DM exhibited elevated blood glucose levels (HbA1c, FBS, and PPBS) and MDA, along with decreased TAC, indicating an increase in oxidative stress. Furthermore, a strong negative correlation was observed between HbA1c and TAC in the diabetic group, suggesting that as blood glucose levels rise, oxidative stress also increases.

Key words : Diabetes mellitus, blood glucose, total antioxidant capacity, malondialdehyde.

[1*]Associate Professor, [2]Professor, [3]Assistant Professor, [4]Assistant Professor

Diabetes mellitus is a persistent metabolic disorder characterized by impaired glucose, lipid, and protein metabolism due to defects in insulin secretion (beta cell dysfunction) or action (insulin resistance) or both[4]. The global rise in the prevalence of diabetes can be attributed to changes in human lifestyle and behavior over the past century[30]. These changes have resulted in various risk factors for diabetes, including obesity, a sedentary lifestyle, stress, smoking, alcohol consumption, polycystic ovary syndrome, mental health conditions, and sleep disturbances[2]. Individuals with diabetes may face long-term complications such as diabetic nephropathy, neuropathy, retinopathy, and cardiovascular diseases, which not only significantly impact their health but also place a substantial economic burden on society[21,25].

Currently, there are 415 million people worldwide living with diabetes, and this number is projected to exceed 642 million by the year 2040. In India alone, the prevalence of diabetes mellitus is 8.7%, affecting approximately 69.2 million individuals, and it is estimated to reach 109 million by 2035 [15,16].

Excessive accumulation of reactive free radicals, caused by an imbalance between the formation and destruction of ROS and RNS, leads to oxidative stress. While ROS and RNS act as signaling molecules in normal conditions, their excessive presence under oxidative conditions can lead to cellular damage. This damage occurs through harmful reactions with proteins, lipids, and DNA, ultimately contributing to the development of oxidative stress-related diseases such as diabetes, obesity, Alzheimer's disease (AD), and Parkinson's disease (PD)[12,27,29].

Antioxidants present in cells, like glutathione, along with antioxidant enzymes such as superoxide dismutase (SOD), catalase (XAT), and glutathione peroxidase (GPx), work in tandem to regulate the cellular redox status by neutralizing harmful ROS and RNS[14,19].

Unregulated diabetes mellitus can stem from non-enzymatic protein glycation, glucose auto-oxidation, and activation of the polyol pathway, which ultimately leads to heightened oxidative stress and sustained elevated blood glucose levels[3,23]. Oxidative stress is a key factor in the advancement of diabetes mellitus and its associated complications[6,7,28]. TAC measurement is an important marker for assessing the risk associated with free radical activity in individuals with type-2 diabetes mellitus[1]. On the other hand, MDA is a stable byproduct that is formed due to lipid peroxidation. Numerous studies have been conducted to demonstrate the increased oxidative stress in diabetes mellitus by measuring MDA levels. Therefore, the main objective of this ongoing research is to evaluate the extent of oxidative stress in individuals with diabetes by measuring two markers: TAC and MDA.

A research study was undertaken with type 2 diabetic individuals at School of Allied Healthcare and Science, Malla Reddy University, Hyderabad, and Telangana. Before starting the project, permission was granted by the institutional ethical committee. This study, which took place between January 2021 and July 2021, was a joint effort between the Department of Biochemistry, General Medicine, and Endocrinology. Participants were chosen based on specific criteria for inclusion and exclusion.

Inclusion criteria :

- ✓ Type 2 diabetes mellitus patients of both sexes
- ✓ Duration of diabetes mellitus 5 years or more
- ✓ Patients with age group between 30-60 years
- ✓ DM patients who were willing to give written informed concern
- ✓ Healthy individual for control group

Exclusion criteria :

- ✓ Type-1 DM patients.
- ✓ Patients who were not willing to give their written informed concern
- ✓ Smokers, alcoholics/ pregnant/ lactating women
- ✓ Patients who are receiving antioxidants like vitamin A, E and C and lipid lowering drugs.
- ✓ Patients who were having psychiatric problems
- ✓ Patients with chronic diseases like kidney, liver, cardiac problems, TB, leprosy, recent trauma, surgery.

In the current study, a total of 60 patients diagnosed with type 2 DM who were attending the OPD of General Medicine or Endocrinology, along with 60 healthy individuals, were included based on specific inclusion and exclusion criteria. The socio demographic information and medical history of each patient were collected. Subsequently, under sterile conditions, 5 ml of venous blood was obtained from each participant in both groups and sent to the laboratory for the evaluation of blood glucose parameters, including HbA1c, FBS, and PPBS, as well as oxidative stress markers such as MDA and TAC.

Methods for determination of Biochemical parameters:

- ➢ Malondialdehyde (MDA) was estimated as thiobarbituric acid reactive substances (TBARS).
- ➢ Total antioxidant capacity was analyzed by ferric reducing ability of plasma (FRAP).
- ➢ Blood Glucose was estimated by glucose oxidase and Peroxidase enzymatic method[5].
- ➢ HbA1c was estimated by Ion exchange resin method[26].

Statistical analysis :

The data underwent analysis through an unpaired test, while the correlation between the variables was assessed using Pearson's correlation analysis with SPSS software version 23.0. The findings were presented as mean ± SD (standard deviation), with a P value <0.05 indicating statistical significance.

In the current study, a total of 120 participants were included. Among them, 60 individuals were diagnosed with type 2 DM, while the remaining 60 were healthy subjects. Out of the 60 diabetic patients, 36 were male and 24 were female. The majority of diabetic patients (n=33) were in the age group of 41–50 years, followed by 18 in the age group of 51–60 years, and 9 in the age group of 31–40 years. The BMI of control and diabetic patients was 22.6 ± 0.4 and 24.0 ± 0.5, respectively, with a statistically significant mean difference ($p<0.05$). Table-1 Statistical analysis showed no significant differences in food habits, family history of diabetes, or marital status between group 1 and group 2 participants ($p > 0.05$).

When comparison was made between the groups, diabetic patients showed a significant rise in mean FBS (127.7± 2.87), PPBS (206.1±5.02), and HbA1c (8.27 ±0.35) than the control group (FBS: 85.1 ±3.57; PPBS: 136.3 ± 3.65; HbA1c: 5.25 ± 0.143). The mean difference of study variables was statistically significant (<0.05). Table-1

Table-1. Blood glucose level in control and study group

Variables	Control group Mean± SD	Test group Mean ±SD	Mean difference	P value
FBS (mg/dl)	85.1 ±3.57	127.7± 2.87	-42.6± 1.44	0.000
PPBS (mg/dl)	136.3 ±3.65	206.1±5.02	69.8± 1.96	0.000
HbA1c (%)	5.25 ± 0.14	8.27 ±0.35	-3.02 ±0.12	0.000
BMI (kg/m^2)	22.6 ± 0.4	24.0 ± 0.5	-1.4±0.1	0.000

P value <0.05 statistically significant**

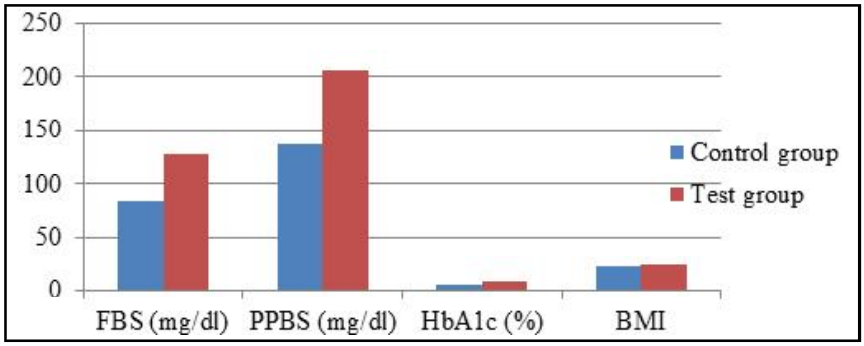

Figure-1. Graphical representation of BMI and blood glucose level in control and test group

Diabetic patients exhibited higher levels of MDA (5.93 ± 0.34) and lower levels of TAC (0.53 ± 0.10) compared to healthy individuals (MDA: 3.22 ± 0.168; TAC: 1.46 ± 0.15). These differences were found to be statistically significant (p<0.05). Table-2 In the diabetic group, there was a positive correlation between HbA1c and MDA (R value: 0.9893; P value: 0.000), as well as a negative correlation between HbA1c and TAC (R value: 0.9243; P value: 0.000). These correlations were also statistically significant (p<0.05). Table-3 and Figures 3 and 4

Table-2. MDA and TAC level in control and study group

Variables	Control group Mean± SD	Test group Mean ±SD	Mean difference	P value
MDA(mmol/l)	3.22 ± 0.168	5.93 ± 0.34	-2.71±0.12	0.000
TAC(mmol/l)	1.46 ± 0.15	0.53 ±0.10	0.93 ±0.05	0.000

P value <0.05** statistically significant

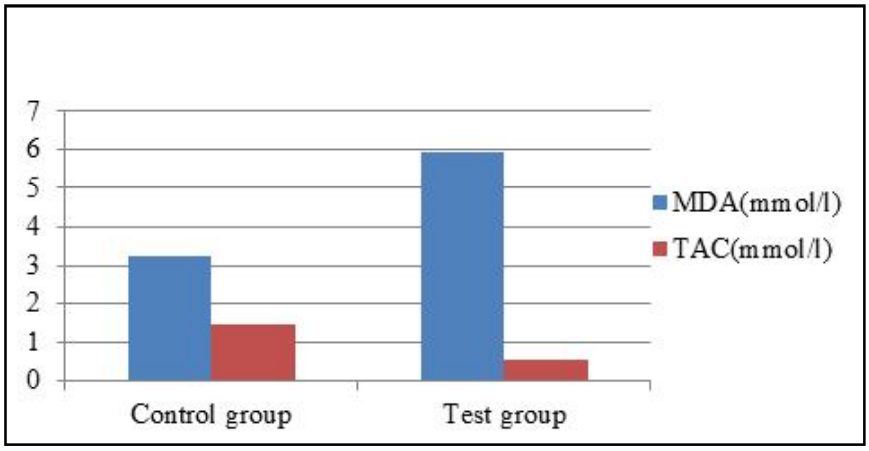

Figure-2. Graphical representation of MDA and TAC level in control and test group

Table-3. Correlation between HbA1c and TAC in diabetic patients

	HbA1c (%)	TAC(mmol/l)	R value	P value
Diabetic patients	8.27 ±0.35	0.53 ±0.10	0.9243	0.000

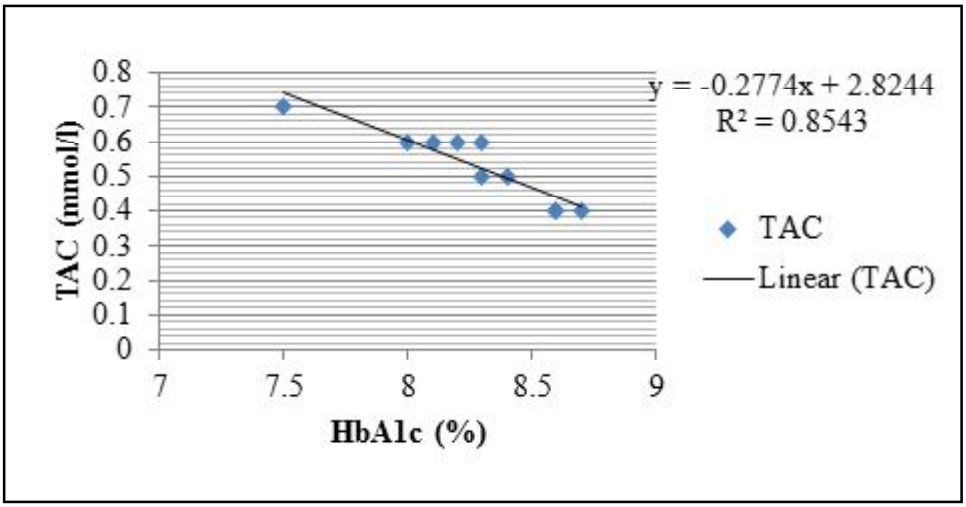

Figure-3. Showing correlation between HbA1c and TAC in diabetic patients

Table-4. Correlation between HbA1c and MDA in diabetic patients

	HbA1c (%)	MDA(mmol/l)	R value	P value
Diabetic patients	8.27 ±0.35	5.93 ± 0.34	0.9893	0.000

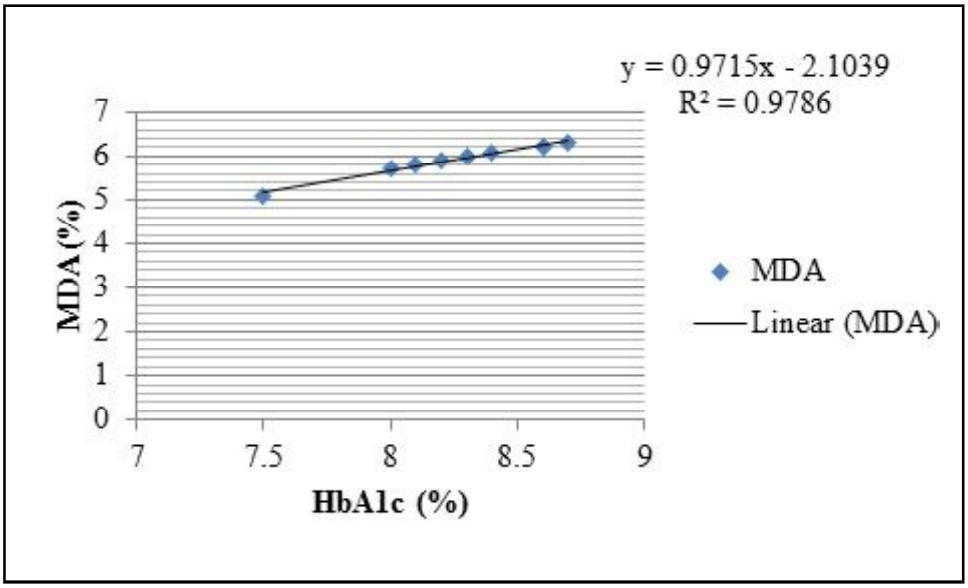

Figure-4. Showing correlation between HbA1c and MDA in diabetic patients

Oxidative stress is characterized by the accumulation of reactive free radicals due to an imbalance between the production and elimination of ROS and RNS. It plays a significant role in the progression of diabetes mellitus and its associated complications. Therefore, this study aimed to evaluate the level of oxidative stress in diabetic patients and its correlation with HbA1c levels. In this study, the majority of diabetic patients were males (n=33), which is consistent with a similar study that also reported a high prevalence of diabetes mellitus in males[10]. However, a previous study found a higher prevalence of DM in females[9]. Among the participants in the diabetic group, 55% (n = 33) belonged to the age group of 41–50 years, followed by 30% in the age group of 31–40 years, and 15% in the age group of 51–60 years. These findings are supported by previous studies[9,10]. The extent of oxidative stress tends to increase with age; therefore, patients between the ages of 30 and 60 were included in this study. The mean BMI (kg/m^2) was higher in the diabetic group, which is consistent with another study[17]. table-1 Statistical analysis did not reveal any significant differences in food habits, family history of diabetes, or marital status between group 1 and group 2 participants ($p > 0.05$). Patients with diabetes mellitus showed a rise in mean HbA1c, FBS, and PPBS levels as compared to healthy individuals, which is in line with the results of previous studies ($p<0.05$)[17,20,24].

In the present study, the degree of oxidative stress was assessed using two parameters, namely TAC and MDA. Individuals with DM exhibited a significant increase in average MDA levels and a decrease in average TAC levels in comparison to healthy subjects ($P<0.05$). table-2 These results were consistent with those of prior research[1,11,22].

As oxidative stress intensifies, MDA levels rise while TAC levels decline. Upon examining the correlation between mean MDA and TAC levels with HbA1c, a strong positive correlation was observed between MDA and HbA1c (R value: 0.9893; p value: 0.000), whereas a markedly negative correlation was found between TAC and HbA1c (R value: 0.9243; p value: 0.000). table 3 and 4 Similar outcomes were reported in a previous study[24].

The decrease in TAC levels in diabetic patients may be attributed to the excessive production of free radicals[13], auto-oxidation of glucose, non-enzymatic glycation[8], and the poor socioeconomic status of individuals. This poor socioeconomic status leads to a reduced intake of dietary antioxidants such as vitamin C, vitamin E, beta-carotene, and sulfur-containing amino acids like methionine. Additionally, it results in a lower availability of micronutrients like selenium, zinc, copper, and manganese, which in turn causes inadequate synthesis of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase[18].

The present study establishes that individuals with diabetes mellitus exhibit elevated blood glucose levels and oxidative stress, coupled with a reduction in total antioxidant capacity. Furthermore, a positive correlation between oxidative stress and glycemic levels was observed. This study suggests that evaluating antioxidant levels and integrating antioxidant-rich elements into treatment strategies can improve glycemic regulation and mitigate the occurrence of diabetic complications.

We would like to acknowledge Department of Biochemistry for the technical support provided for this study.

References :

1. Akinosun OM, and EB. Bolajoko (2007). Total Antioxidant Status in Type 2 Diabetic Patients: Experience at University College Hospital (Uch) Ibadan, Nigeria. Nigerian Journal of Clinical Practice. *10*(2): 126-129.
2. Alexandra Kautzky-Willer, Jürgen Harreiter, and Giovanni Pacini (2016). *Endocr Rev. 37* (3): 278-316.
3. Aybek H, Z Aybek, S Rota, N Sen and M. Akbulut (2008). *Fertil Steril, 90:* 755-760.
4. Banerjee M, and P. Vats (2014). Redox *Biolog. 2:* 170-177.
5. Barham D, Trinder (1972). *Panalyst, 97*(1): 142.
6. Baynes JW. (1991). *Diabetes, 40:* 405-412.
7. Ceriello A. (2000). *Metabolism. 49:* 27-29. 12.
8. Ceriello A, A Quatraro, and D. Giugliano (1992). *Diabetic Med. 9:* 297 299.
9. Chaganti Nagesh, Anil Kumar, Srawan Kumar, and Nilam Nigam (2021). *J Adv Sci Res, 12*(2): 171-177.
10. Chaganti Nagesh, Nilam Nigam, Srawan Kumar, and Anil Kumar (2021). *J adv sci res, 12*(1): 66-71.
11. Chavan VU, and RR. Melinkeri (2013). *Natl J Community Med, 4*(2): 294-299.
12. Fialkow, L., Y. Wang, and G.P. Downey, (2007). *Free. Radic. Biol. Med. 42:* 153–164.
13. Giugliano D, A Ceriello and G. Paolisso (1996). *Diabetes Care. 1*(3): 257-267.
14. Ji, T., L. Zheng, J. Wu, M. Duan, Q. Liu, P. Liu, C. Shen, J. Liu, Q. Ye and J. Wen, *et al*. (2023). *Nat. Commun. 14:* 2807.
15. Joshi SR, and RM. Parikh (2007). *J Assoc Physicians India. 55:* 323–4.

16. Kumar A, MK Goel, RB Jain, P Khanna, and V. Chaudhary (2013). *Australas Med J. 6*(10): 524-31.
17. Kumar MN, A Kumar and A. Pallavi (2020). *Al Ameen J Med Sci. 13*(4): 242-247.
18. Low PA. (1997). *Diabetes. 46*(2): 538-542.
19. Mates, J.M., C. Perez-Gomez and I.N. De Castro, (1999). *Clin. Biochem. 32:* 595–603.
20. Naresh Kumar M, A Pallavi and A. Kumar (2020). *Al Ameen J Med Sci 13*(2): 83-87.
21. Rahimi R, S Nikfar, B Larijani, and M. A Abdollahi (2005). *Biomed Pharmacother. 59*(7): 365-373.
22. Sai Ravi Kiran B, T Mohana Lakshmi, R Srikumar, and E. Prabhakar Reddy (2016). *Int. J. Pharm. Sci. Rev. Res., 40*(1): 271-277.
23. Sozmen EY, B Sozmen, Y Delen, and T. Onat (2001). *Arch Med Res. 32:* 283-287.
24. Suvarna Devi C, Nishitha Ganugapeta, Sai Sravan Kumar, Reddythala, and M. Anuswaru (2024). *Hba1c. Journal of cardiovascular disease research. 15*(05): 431-438.
25. Tiwari BK, KB Pandey, AB Abidi, and SI. Rizvi (2013). *J Biomark. 37:* 87-90.
26. Trivelli LA, Ranney HM, Lai. (1971). *New Eng. J. med,* 284-353
27. Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, (2006). *Int. J. Biochem. Cell Biol., 39:* 44–84.
28. Wolff SP, and RT. Dean (1987). *Biochem J. 245:* 243 250.
29. Zhang, J., J. Hu, W. Sang, J. Wang, and Q. Yan, (2016). *ACS Macro Lett. 5:* 919–924.
30. Zimmet P, KG Alberti, and J. Shaw (2011). *Nature. 414:* 782-787.