

Original Article

## Estimation Of Total Antioxidant Capacity In Patients Of Type-2 Diabetes Mellitus In Correlation With Hba1c

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### ABSTRACT

**Introduction;** Diabetes mellitus is a metabolic disorder resulting from insufficient insulin production or decreased insulin effectiveness, or both. The development of diabetic complications is often attributed to oxidative stress, which arises from an imbalance between the production and elimination of free radicals.

**Aim:** To assess the extent of oxidative stress in individuals with type-2 diabetes mellitus in comparison to healthy individuals.

**Materials and methods:** A total of 140 participants were enrolled in this study, with 70 participants being healthy individuals (group A) and the remaining 70 participants were diagnosed with type 2 DM (group B). Blood samples were collected from each participant to measure various parameters related to blood glucose, including glycosylated hemoglobin (HbA1c %), fasting blood sugar (FBS mg/dl), postprandial blood sugar (PPBS mg/dl), as well as oxidative stress markers such as malondialdehyde (MDA) and total antioxidant capacity.

**Results:** Upon conducting a comparison between the groups, it was noted that individuals with diabetes mellitus (group B) demonstrated higher levels of HbA1c, FBS, PPBS, and MDA, while the healthy participants (group A) exhibited higher levels of Total Antioxidant Capacity. This disparity was found to be statistically significant ( $p < 0.05$ ).

**Conclusion:** The present study findings indicate that individuals with diabetes mellitus exhibited elevated blood glucose levels and oxidative stress. Additionally, there was a significant reduction in total antioxidant capacity. When examining the correlation with HbA1c, a strong negative correlation was observed. It is suggested that increased oxidative stress may contribute to diabetic complications.

**Key words:** Total antioxidant capacity (TAC) Malondialdehyde (MDA) Diabetes mellitus (DM)

## INTRODUCTION

Diabetes mellitus is a metabolic disorder that occurs as a result of insufficient insulin, impaired insulin action, or both. This condition is characterized by a persistent increase in blood sugar levels.<sup>1</sup> DM affects the metabolism of carbohydrates, lipids, and proteins. The main causes of all forms of diabetes are the dysfunction or destruction of insulin-secreting cells in the pancreas and insulin receptor resistance. In India, diabetes is rapidly becoming a potential epidemic, with over 62 million individuals currently diagnosed with the disease. It is predicted that the diabetic population in India will reach 109 million by 2035.<sup>2, 3</sup> The global increase in diabetes incidence can be attributed to changes in human lifestyle and behavior over the last century.<sup>4</sup>

Oxidative stress refers to the imbalance between the production and removal of free radicals, specifically reactive oxygen species (ROS) and reactive nitrogen species (RNS), which leads to their accumulation in the body.<sup>5</sup> This accumulation of ROS and RNS is a significant factor in the development of diabetes complications and insulin resistance.<sup>6,7,8</sup> While ROS and RNS serve as signaling molecules at normal physiological levels, excessive amounts of these molecules can cause cellular damage by reacting with proteins, lipids, and DNA. This damage contributes to the pathogenesis of various oxidative stress-related diseases, including diabetes, obesity, neurological diseases like Alzheimer's disease and Parkinson's disease, rheumatoid arthritis, and even cancer.<sup>9-12</sup> Insufficient insulin production, hindered proinsulin vesicle integration into the plasma membrane, and decreased exocytosis in response to glucose are all consequences of oxidative stress. This stress can also trigger apoptotic processes in pancreatic cells, resulting in the death and loss of beta cells. To maintain a balanced cellular redox status, cellular antioxidants like glutathione and antioxidant enzymes such as superoxide dismutase (SOD), catalase (XAT), and glutathione peroxidase (GPx) work to sequester reactive oxygen species (ROS) and reactive nitrogen species (RNS).<sup>16, 17</sup>

The total antioxidant capacity (TAC) is a cumulative parameter that takes into account all antioxidants found in serum/plasma, foods (dietary total antioxidant capacity), and other body fluids<sup>18, 19, 20</sup>. Measurement of TAC provides a useful indicator of the risk associated with free radical activity in individuals with type 2 diabetes.<sup>21</sup>

The breakdown of Poly Unsaturated Fatty Acids (PUFA) results in the production of MDA, a stable byproduct of lipid peroxidation. Numerous studies have provided evidence supporting the idea of increased oxidative stress in diabetes mellitus through the measurement of MDA. Therefore, this current study aimed to assess the level of oxidative stress in diabetic patients by utilizing two parameters: TAC and MDA.

## MATERIALS & METHODS

The current research involved a hospital-based comparative study on type 2 diabetic patients at Narayana Medical College and Hospital in Nellore, AP. The study was conducted in the biochemistry department in collaboration with the general medicine department from January 2021 to July 2021. Before commencing the study, approval was obtained from the institutional ethical

committee. Participants were selected based on specific inclusion and exclusion criteria for the study.

#### **Inclusion criteria:**

- Type 2 diabetes mellitus patients of both sexes
- Patients with age group between 30-60 years
- Patients who were willing to give written informed concern

#### **Exclusion criteria;**

- Smokers / alcoholics/ pregnant & lactating women
- Patients of Type-1 diabetes mellitus
- Unwilling to give informed concern and participate in present study
- Mental incapacity to follow the instructions
- Patients with psychiatry, liver, kidney, cardiac problems and also chronic infections like TB, leprosy, recent trauma, surgery.
- Patients who are receiving antioxidants like vitamin A, E and C

A total of 140 individuals were enrolled in the study, consisting of 70 participants being healthy individuals (group A) and the remaining 70 participants were diagnosed with type 2 DM (group B). Demographic information and medical history were obtained from all participants at the start of the study. Followed by 5 ml of venous blood was collected from each participant and sent to the laboratory for analysis of blood glucose levels (FBS, PPBS and HbA1c), as well as oxidative stress markers (MDA and TAC).

#### **Determination of Biochemical parameters:**

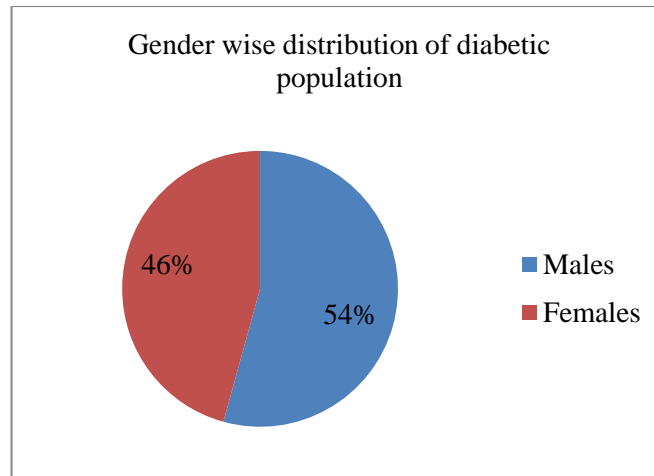
Glucose oxidase and Peroxidase enzymatic method were used to estimate fasting blood sugar and postprandial blood sugar, while Ion exchange resin method was used to estimate HbA1c. Malondialdehyde (MDA) was estimated as thiobarbituric acid reactive substances (TBARS), and Total antioxidant capacity was analyzed by ferric reducing ability of plasma (FRAP).

#### **Statistical analysis:**

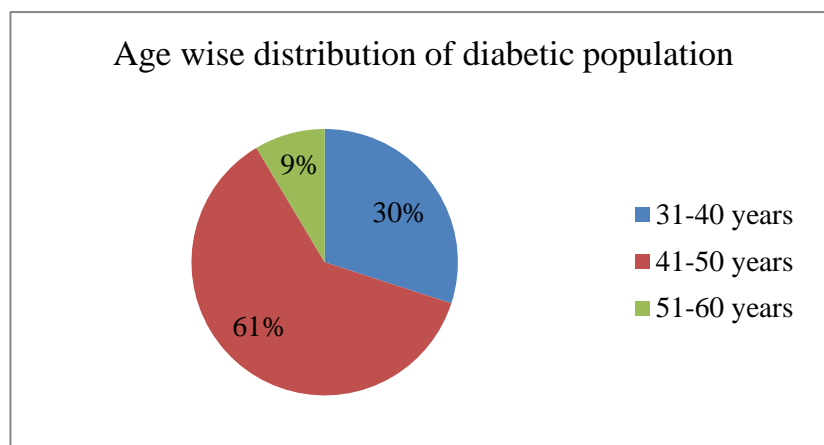
The data was gathered and inputted into MS Excel. The obtained data was analyzed by an unpaired test and the correlation between TAC and HbA1c was done using Pearson's correlation analysis using SPSS software version 23.0. The results were presented as mean  $\pm$  standard deviation, with a p-value of  $<0.05$  indicating statistical significance.

## **RESULTS**

In this study, a group of 70 individuals with diabetes and 70 individuals without diabetes were included. Out of the 70 diabetic patients, 38 were male and 32 were female. **Figure-1** The majority of the diabetic population, 61.42% (n = 43), fell within the age range of 40–50 years. Additionally, 30% (n = 21) of the diabetic patients were between 30-40 years old, while the remaining 8.57% (n = 6) were aged 51–60 years. **Figure-2**



**Figure-1:** Gender wise distribution of diabetic population



**Figure-2:** Age wise distribution of diabetic population

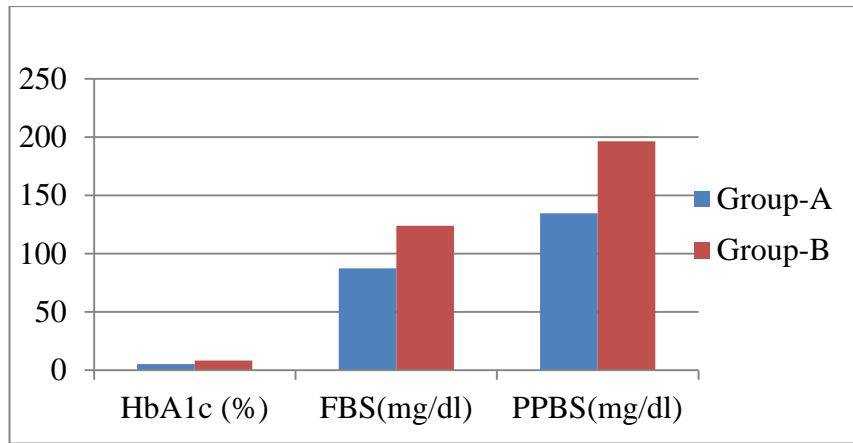
Patients diagnosed with type-2 DM exhibited elevated levels of HbA1c ( $8.12 \pm 0.31$  %), FBS mg/dl ( $123.8 \pm 36.86$  mg/dl), and postprandial blood sugar ( $196.5 \pm 16.61$  mg/dl) compared to the control group (HbA1c:  $5.309 \pm 0.25$ ; FBS:  $87.45 \pm 2.16$ ; PPBS:  $134.5 \pm 4.48$ ). The differences in means were found to be statistically significant ( $p < 0.05$ ). **Table-1**

When comparison was made between the two groups, it was found that patients with type 2 DM (group B) had higher levels of MDA ( $6.41 \pm 0.31$  (mmol/l)) and lower levels of TAC ( $0.62 \pm 0.12$  (mmol/l)) compared to the healthy subjects (MDA:  $3.38 \pm 0.28$  (mmol/l); TAC:  $1.37 \pm 0.20$  (mmol/l)). The difference in means was statistically significant ( $p < 0.05$ ). **Table-2**

**Table-1:** Comparison of blood glucose level subjects with diabetes mellitus and controls

Variable	Group-A (Mean $\pm$ SD)	Group-B (Mean $\pm$ SD)	Mean difference	P value
HbA1c (%)	$5.309 \pm 0.25$	$8.12 \pm 0.31$	$2.81 \pm 0.12$	0.000
FBS(mg/dl)	$87.45 \pm 2.16$	$123.8 \pm 36.86$	$36.36 \pm 11.13$	0.004
PPBS(mg/dl)	$134.5 \pm 4.48$	$196.5 \pm 16.61$	$62 \pm 5.18$	0.000

**$P < 0.05^{**}$  statistically significant**

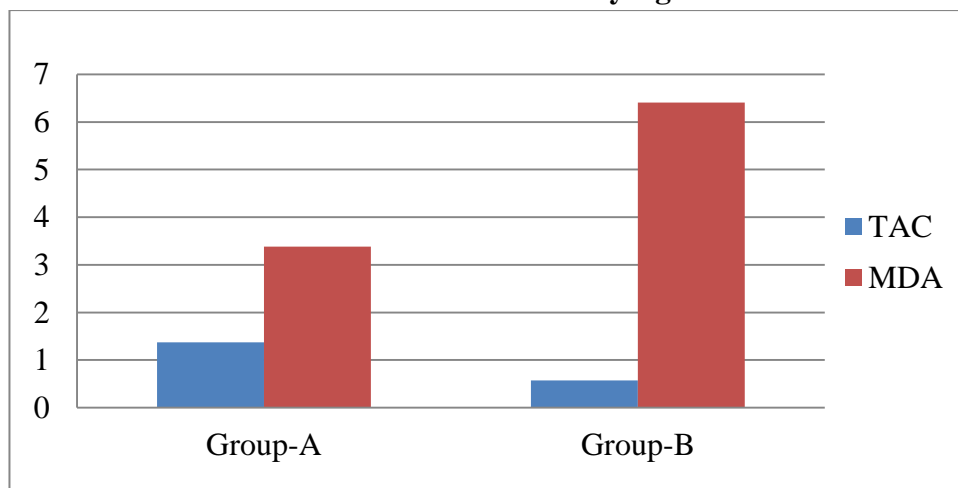


**Figure -3:** Graphical representation of blood glucose level in healthy subjects and DM patients

**Table-2; Comparison of study variables in subjects with diabetes mellitus and controls**

Variable	Group-A (Mean± SD)	Group-B (Mean± SD)	Mean Difference	P value
TAC (mmol/l)	1.37± 0.20	0.57± 0.16	0.8 ± 0.07	0.000
MDA(mmol/l)	3.38± 0.28	6.41± 0.31	3.03± 0.12	0.000

**P<0.05\*\*= statistically significant**

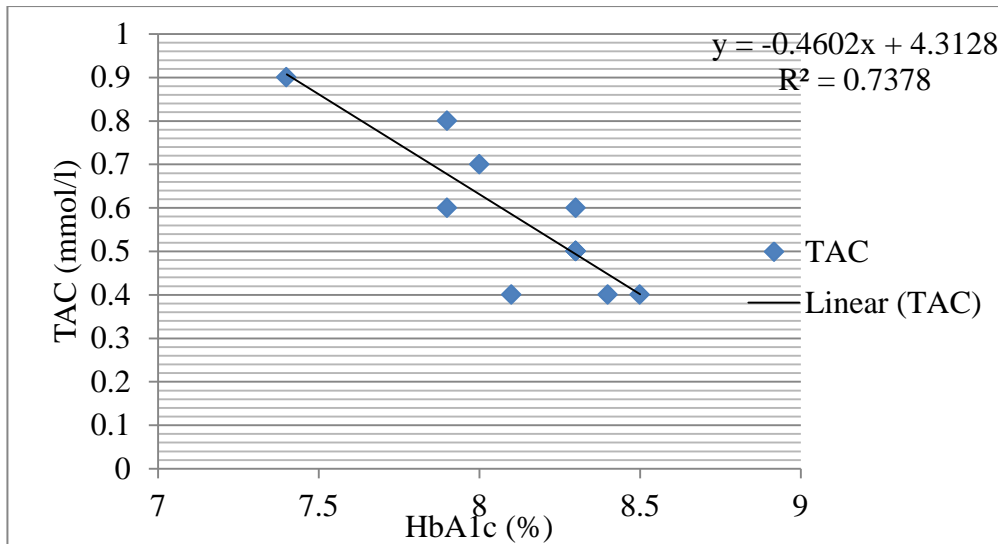


**Figure-4:** Graphical representation of MAD and TAC in healthy subjects and DM patients

A strong negative correlation was found between HbA1c and total antioxidant capacity in diabetic patients through Pearson’s correlation analysis, with statistical significance (r value= -0.8589, p value =< .0001). **Table 3**

**Table-3:** Correlation between Total antioxidant capacity and HbA1c in diabetic patients

	HbA1c (%)	TAC(mmol/l)	R value	P value
Diabetic patients (group-B)	8.12± 0.31	0.62 ± 0.12	-0.858	0.000



**Figure-5:** Correlation between Serum TAC and HbA1c in diabetic patients

## DISCUSSION

The study aimed to assess the oxidative stress levels in individuals with type-2 diabetes compared to healthy individuals. The majority of participants in the diabetic group were males, primarily falling within the 40-50 age range. As oxidative stress tends to increase with age, the study focused on individuals aged between 30 to 60 years. **Figure-1&2** In this study, patients with diabetes mellitus exhibited notably higher levels of HbA1c (%), FBS (mg/dl), and PPBS (mg/dl) in comparison to the healthy subjects. The mean differences in HbA1c, FBS, and PPBS between the two groups were found to be statistically significant ( $P < 0.05$ ). **Table-1& figure-3**

The current study revealed a significant increase in mean MDA levels among type 2 diabetes mellitus patients compared to healthy subjects ( $p < 0.05$ ). Similar findings were reported in other studies.<sup>22-24</sup> MDA is a stable end product of lipid peroxidation resulting from the decomposition of Poly Unsaturated Fatty Acids (PUFA). Numerous studies have supported the notion of heightened oxidative stress in diabetes mellitus through MDA estimation.

The TAC levels were significantly lower in diabetic patients compared to healthy subjects ( $p < 0.05$ ). This finding is consistent with previous studies.<sup>21, 25, 24</sup> When correlating total antioxidant capacity with HbA1c in diabetic patients using Pearson's correlation analysis, a strong negative correlation was observed ( $r$ -value =  $-0.8589$ ,  $p$ -value =  $< .0001$ ). **Table 3 and figure-4** The decrease in TAC levels was associated with an increase in HbA1c levels. A decrease in TAC levels and an increase in MDA levels indicate oxidative stress, leading to poor glycemic control and an increased risk of developing diabetic complications.

The total antioxidant capacity (TAC) encompasses all antioxidants found in serum/plasma, foods (dietary total antioxidant capacity), and other bodily fluids.<sup>26-28</sup> TAC provides a cumulative parameter rather than a simple sum of measurable antioxidants.<sup>29</sup> The measurement of total antioxidant capacity is a valuable indicator of the risk related to free radical activity in individuals with type 2 diabetes.<sup>30</sup>

## CONCLUSION

Findings of the study indicate that individuals with type 2 diabetes have elevated levels of oxidative stress, potentially caused by a decrease in overall antioxidant capacity. A significant negative correlation was observed between HbA1c and total antioxidant capacity, suggesting that increased oxidative stress negatively impacts glycemic control. This study proposes that assessing antioxidant levels and incorporating antioxidant-rich components into treatment plans can enhance glycemic control and prevent diabetic complications.

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