

# Oxidative stress and its relation to glycemic control in patients of type 2 diabetes mellitus

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## Abstract

**Background:** Diabetes mellitus (DM) is one of the most frequent chronic diseases worldwide and is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia.

**Objective:** This study was conducted with an objective to evaluate oxidative stress in patients of DM and correlate it with glycemic control.

**Materials and Methods:** A total of 90 subjects of both sex groups, aged between 45–60 years were enrolled for this study. Of 90 subjects, 60 were patients with type 2 diabetes and 30 were healthy without diabetes. Glycemic status was categorized as good glycemic control if glycated hemoglobin (HbA1c) <7% and poor glycemic control if HbA1c ≥7%. Serum fasting blood sugar (FBS) was measured by Glucose Oxidase-Peroxidase (GOD-POD) method. Serum malondialdehyde (MDA) was measured by the method described by Kei Satoh. HbA1c was measured by turbidimetric immunoassay.

**Result:** Patients who were diabetic with poor glycemic control have increase in the levels of MDA and HbA1c as compared with patients who were nondiabetic ( $p < 0.0001$ ). The MDA level in diabetic patients with good glycemic control was increased as compared with nondiabetic controls ( $p = 0.0186$ ) whereas the differences of HbA1c level between diabetic with good glycemic control and nondiabetic control were statistically insignificant ( $p = 0.3297$ ). The HbA1c and MDA levels in diabetic patients with poor glycemic control were increased compared with diabetic patients with good glycemic control ( $p < 0.0001$ ). In all the studied groups, MDA was positively correlated with HbA1c.


**Conclusion:** The study suggests that MDA and antioxidants should be measured along with routine parameters of disease and antioxidants should be incorporated in medication given to diabetic patients so that it can counterbalance the oxidative stress produced during diabetes.

**KEY WORDS:** Diabetes mellitus, oxidative stress, glycemic control

## Introduction

Diabetes mellitus (DM) is a group of metabolic disease characterized by hyperglycemia resulting from a defect in insulin secretion, insulin action, or both.<sup>[1]</sup> Some 382 million people worldwide, or 8.3% of adults, are estimated to have

diabetes. About 80% lives in low- and middle-income countries. If these trends continue, by 2035, some 592 million people, or one adult in 10, will have diabetes. This equates to approximately three new cases every 10 seconds or almost 10 million per year. The largest increases will take place in the regions where developing economies are predominant.<sup>[2]</sup> Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in DM.<sup>[3]</sup> Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs, which leads to domination of the condition called oxidative stress.<sup>[4]</sup>

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Malondialdehyde (MDA) is an organic compound with the formula  $\text{CH}_2(\text{CHO})_2$ . This reactive species occurs naturally and is a marker for oxidative stress. ROS degrade polyunsaturated lipids present on cell membrane forming MDA. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism.<sup>[4]</sup>

Glycated hemoglobin (HbA1c) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over a prolonged period. It is formed in a nonenzymatic pathway by normal exposure of hemoglobin to high plasma levels of glucose. Glycation of hemoglobin had been associated with cardiovascular diseases, nephropathy, and retinopathy in DM.<sup>[5]</sup> HbA1c is effective in monitoring long-term glucose control in people with DM.<sup>[6]</sup> It has been shown that elevated HbA1c is associated with an increased risk of complications in patients with type 2 DM and that lowering of HbA1c reduces such risk. Thus, HbA1c serves as a surrogate for the risk of microvascular and macrovascular complications, and these results firmly establish HbA1c as a useful measure of long-term glycemic control.<sup>[7]</sup> This study was conducted with an objective to evaluate oxidative stress in patients of DM and correlate it with glycemic control.

## Materials and Methods

This research work was carried out in the Department of Biochemistry, Muzaffarnagar Medical College and Hospital, Muzaffarnagar. The cases were selected from those who attended the medicine outpatient department during the period of 1 year (September 2014 to August 2015) at Muzaffarnagar Medical College and Hospital, Muzaffarnagar. The investigations were carried out in the Biochemistry Laboratory, Muzaffarnagar Medical College and Hospital, Muzaffarnagar.

### Subjects

A total of 90 subjects of both sex groups, aged between 45–60 years were enrolled for this study. Of the 90 subjects, 60 were type 2 diabetic patients and 30 were healthy nondiabetic individuals. Healthy nondiabetic individuals serve as controls for this study and were selected from general population of the same region. Participants were informed about the study. Informed consent was taken from each subject.

### Exclusion Criteria

The individuals having hepatic disease, cardiovascular disease, any chronic or acute inflammatory illness, all types of cancer, pulmonary tuberculosis, addiction to alcohol, who smoke, and having prolonged illness were excluded from the study.

### Glycemic Status

Glycemic status was categorized as good glycemic control if HbA1c <7% and poor glycemic control if HbA1c ≥7%.

## Collection of Blood Sample

About 5 mL of blood was drawn after an overnight fast under aseptic precautions from clinically diagnosed type 2 DM patients and controls, and divided into 2 tubes, marked as 1 and 2.

1. Test tube 1 contains 3 mL of blood with no anticoagulant and blood was allowed to clot and serum was separated. Serum was used for measurement of blood sugar and MDA.
2. Test tube 2 contains 2mL of blood, with Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant, which was used for estimation of HbA1c.

## Parameters Measured

The following parameters were measured in this study:

1. serum fasting blood sugar (FBS);
2. serum MDA;
3. HbA1c.

Serum FBS was measured by GOD-POD method with the help of CPC TurboChem100, a fully automatic analyzer. Serum MDA was measured by the method described by Kei Satoh.<sup>[8]</sup> HbA1c was measured by turbidimetric immunoassay with the help of CPC TurboChem100.

## Statistical Analysis

Results were statistically analyzed by “GraphPad Quick-Calcs *t*-test calculator.” Student’s *t*-test was used to assess the significance of difference between the groups. All results were presented as mean ± standard deviation (SD). A *p*-value of less than 0.05 was considered significant.

## Result

Based on HbA1c level, of the 60 subjects with diabetes, 30 patients had poor glycemic control whereas remaining 30 had good glycemic control.

Diabetic patients with poor glycemic control have increase in the levels of MDA and HbA1c as compared with nondiabetic control and were statistically highly significant ( $p < 0.0001$ ). The MDA level in diabetic patients with good glycemic control was increased as compared with nondiabetic control and was statistically significant ( $p = 0.0186$ ) whereas the differences of HbA1c level between diabetic patients with good glycemic control and nondiabetic control were statistically insignificant ( $p = 0.3297$ ). The HbA1c and MDA levels in diabetic patients with poor glycemic control were increased compared with diabetic patients with good glycemic control and the differences were statistically highly significant ( $p < 0.0001$ ). In all the studied groups, MDA was positively correlated with HbA1c.

## Discussion

There has been considerable interest in the concept that the uncontrolled lipid peroxidation is a key contributing factor

**Table 1:** The mean and standard deviation of FBS, HbA1c, and MDA in type 2 diabetic patients with poor glycemc control and nondiabetic patients

Parameters	Diabetic patients with poor glycemc control	Nondiabetic control	p-Value
FBS (mg/dL)	174.77 ± 27.61	87.10 ± 5.93	0.0001 S
HbA1c (%)	8.71 ± 0.84	5.58 ± 0.40	0.0001 S
MDA (nmol/mL)	4.72 ± 0.12	3.65 ± 0.17	0.0001 S

FBS, fasting blood sugar; HbA1c, glycmated hemoglobin; MDA, malondialdehyde; S, significant.

**Table 2:** The mean and standard deviation of FBS, HbA1c, and MDA in type 2 diabetic patients with good glycemc control and nondiabetic patients

Parameters	Diabetic patients with good glycemc control	Nondiabetic control	p-Value
FBS (mg/dL)	90.07 ± 6.98	87.10 ± 5.93	0.0814 NS
HbA1c (%)	5.70 ± 0.56	5.58 ± 0.40	0.3297 NS
MDA (nmol/mL)	3.76 ± 0.15	3.65 ± 0.17	0.0186 S

FBS, fasting blood sugar; HbA1c, glycmated hemoglobin; MDA, malondialdehyde; NS, nonsignificant; S, significant.

**Table 3:** The mean and standard deviation of FBS, HbA1c, and MDA in type 2 diabetic patients with poor glycemc control and good glycemc control

Parameters	Diabetic patients with poor glycemc control	Diabetic patients with good glycemc control	p-Value
FBS (mg/dL)	174.77 ± 27.61	90.07 ± 6.98	0.0001 S
HbA1c (%)	8.71 ± 0.84	5.70 ± 0.56	0.0001 S
MDA (nmol/mL)	4.72 ± 0.12	3.76 ± 0.15	0.0001 S

FBS, fasting blood sugar; HbA1c, glycmated hemoglobin; MDA, malondialdehyde; S, significant.

**Table 4:** Correlation coefficient (*r*) of MDA with HbA1c in studied groups

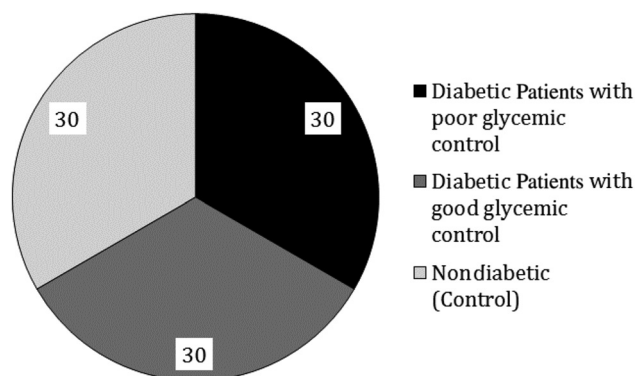
Groups	Parameter	HbA1c
Diabetic patients with poor glycemc control	MDA	0.9146*
Diabetic patients with good glycemc control		0.7008*
Nondiabetic control		0.7160*

HbA1c, glycmated hemoglobin; MDA, malondialdehyde.

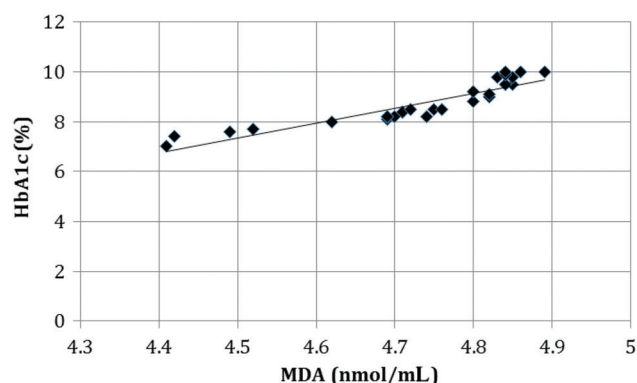
\*<0.05 = Significant.

in the pathophysiology of coronary artery disease, especially in type 2 diabetes. Lipid peroxidation eventually is a sequence of the injury caused by ROS. Free radical damages can accumulate over time and may thereby contribute to cell injury and development of human diseases. Free radicals have been implicated in the development of several diseases including atherosclerosis, diabetes, hypertension, and obesity.<sup>[9,10]</sup> In this study, type 2 diabetic patients with poor glycemc control had significantly higher levels of MDA and HbA1c as compared with nondiabetic control, which is in agreement with the study done by Ikekpeazu et al.<sup>[11]</sup> In this study, HbA1c is used as an index of metabolic control. The increased HbA1c levels

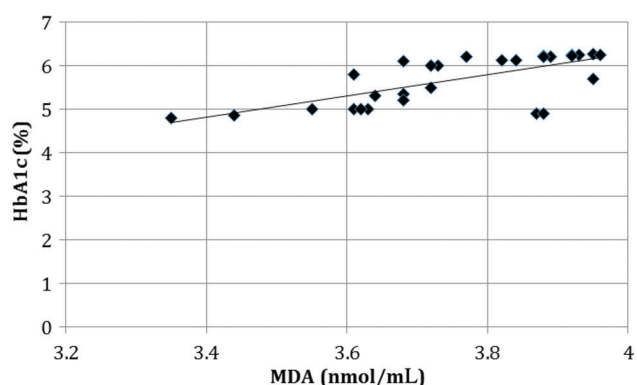
reflect the poor metabolic control of diabetic patients. This study also showed that type 2 diabetic patients with good glycemc control had slightly increased levels of HbA1c and MDA compared with nondiabetic control but the differences were statistically insignificant. Our study is in contrast with the study done by Ikekpeazu et al., who showed that the MDA levels were significantly increased in diabetic patients with good glycemc control as compared with nondiabetic control.<sup>[11]</sup> The result showed that MDA was positively correlated with HbA1c in diabetic patients. This was in agreement with some previous studies.<sup>[6,11]</sup> It is known that hyperglycemia leads to glycosylation of proteins such as Hb, which could also lead



**Figure 1:** Pie chart showing distribution of studied subjects.

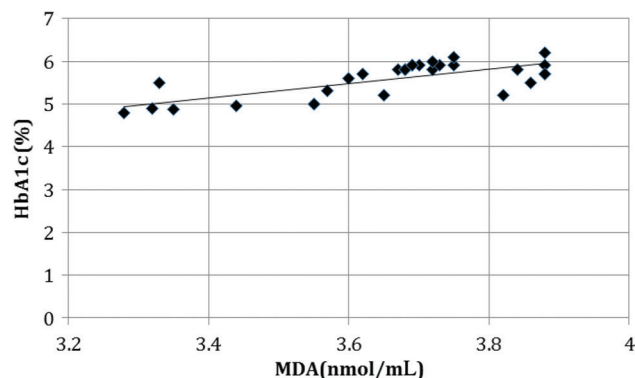


**Figure 2:** Correlation between MDA and HbA1c in diabetic patients with poor glycemc control.



**Figure 3:** Correlation between MDA and HbA1c in diabetic patients with good glycemc control.

to the formation of free radicals because of auto-oxidation of Amadori products.<sup>[12]</sup> This study supports the fact that there are increased levels of FBS and MDA in type 2 DM patients compared with nondiabetic controls and the oxidative stress that is measured as MDA is strictly influenced by glycemc control.



**Figure 4:** Correlation between MDA and HbA1c in nondiabetic control.

## Conclusion

In our study, we concluded that there is a positive correlation of MDA with HbA1c in diabetic patients. So, the study suggests that MDA and antioxidants should be measured as markers of lipid peroxidation along with routine parameters of disease, and antioxidants should be incorporated in medication given to diabetic patients so that it can counterbalance the oxidative stress produced during diabetes. However, further studies with adequate sample size are needed to validate this suggestion.

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## References

1. Moussa SAA. Biophysical changes in red blood cells and hemoglobin components of diabetic patients. *J Genet Eng Biotechnol.* 2007;5(1):27–32.
2. International Diabetes Federation. *IDF Diabetes Atlas*, 6th edn. Brussels, Belgium: International Diabetes Federation, 2013.
3. Ramachandran A, Das AK, Joshi SR, Yajnik CS, Shah S, Kumar KMP. Current status of diabetes in India and need for novel therapeutic agents. *J Assoc Phys India.* 2010;58:7–9.
4. Kshitiz KK, Varun SK, Ranjan A, Kesari JR. Study of serum malondialdehyde and vitamin C status in type 2 diabetes mellitus. *Int J Curr Res Acad Rev.* 2015;3(4):20–25.
5. Reynolds TM, Smellie WS, Twomey PJ. Glycated haemoglobin (HbA1c) monitoring. *BMJ.* 2006;333(7568):586–88.
6. Rani AJ, Mythili SV. Study of oxidative stress and its relation to glycemc control in type 2 diabetes mellitus. *Res J Pharm Biol Chem Sci.* 2014;5(5):589–92.
7. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). United Kingdom prospective diabetes study group. *Lancet.* 1998;352(9131):837–53.

8. Satoh K. Plasma lipid peroxide in cerebrovascular disorder determined by a new colorimetric method. *Clin Chim Acta*. 1978;90(1):37–43.
9. Delanty N, Dichter MA. Oxidative injury in the nervous system. *Acta Neurol Scand*. 1998;98(3):145–53.
10. Bikkad MD, Somwanshi SD, Ghuge SH, Nagane NS. Oxidative stress in type II diabetes mellitus. *Biomed Res*. 2014;25(1):84–7.
11. Ikekpeazu EJ, Neboh EE, Ejezie FE, Ibegbu MD, Ike IE. Oxidative stress and glycaemic control in type 2 diabetic patients in Enugu, South-East Nigeria. *Ann Med Health Sci Res*. 2011;1(1):123–8.
12. Arora M, Mahat RK, Kumar S, Tyagi S, Nehra R, Batra J. Effect of lipid peroxidation on erythrocyte fragility in patients of type 2 diabetes mellitus. *J Diabetes Health*. 2015;108:255–60.

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