

# Diabetes mellitus is metabolic disorders characterized biology essay

[Science](#), [Biology](#)



**WARNING: This Essay Has No Title!**

of paper: Study of protein carbonyl group, nitric oxide and MDA (index of lipid peroxidation) as biomarkers of oxidative stress in type 2 diabetes mellitus.

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**Abstract:**

Diabetes mellitus is metabolic disorders characterized by hyperglycemia and abnormalities in lipid and protein metabolism. The free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its later complications. The present study was planned to study the biomarkers of oxidative stress, such as protein carbonyl (CO) group, nitric oxide in the form of total nitrite (NO<sub>x</sub>) and malondialdehyde (MDA) as an index of lipid

peroxidation in type 2 DM patients and normal individuals. We studied 60 cases of type 2 DM and 30 healthy individuals as control. Serum protein carbonyl estimated by dinitrophenyl hydrazine (DNPH) method, Nitrate and nitrite concentrations in terms of total nitrites (NO<sub>x</sub>) by Griess reaction and MDA by thiobarbituric acid reagent test. We found highly significant increase in the level of protein carbonyl, NO<sub>x</sub> and MDA in type 2 DM patients compared to healthy subjects. From our study it is concluded that there is increased production of ROS and oxidative stress in type 2 DM, which may play major role in the pathogenesis of diabetic complications and supplementation of antioxidants along with anti-diabetic therapy may be beneficial. Keywords: Diabetes mellitus, oxidative stress, protein carbonyl (CO) group, nitric oxide (NO), malondialdehyde (MDA).

### **Introduction:**

Diabetes mellitus (DM) is characterized by absolute or relative deficiencies in insulin secretion and /or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. The free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its later complications<sup>1</sup>. A free radical can be defined as chemical species possessing an unpaired electron. Free radicals can be positively charged, negatively charged or electrically neutral. Free radicals have independent existence and are generally highly reactive<sup>2</sup>. Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense system of an organism<sup>3</sup>. Oxidants are the reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS may

damage all types of biological molecules. In diabetes, there is generation of ROS and oxidative stress causes oxidation of protein leading to formation of protein carbonyl group (CO)<sup>4</sup>. Nitric oxide (NO) is a reactive diatomic gaseous molecule with an unpaired electron (a free radical). Insufficient production of NO has been implicated in the development of hypertension, atherogenesis<sup>5</sup>. Peroxidation of PUFA in lipid membranes severely damages the cell membranes and responsible for damage to tissue where it may be a cause of inflammatory diseases. Malondialdehyde (MDA) and other aldehydes have been identified as products of Lipid peroxidation<sup>6</sup>. The study was aimed to find out concentration of oxidative stress markers, such as protein carbonyl (CO) group, nitric oxide (NO) and malondialdehyde (MDA) in type 2 DM patients compared to healthy individuals.

### **Materials and methods:**

The study was conducted in the department of Biochemistry, B. J. Medical College, and Sassoon General Hospitals, Pune, over a period of one year. Study was approved by ethical committee and informed consent was taken from all participants. We studied 60 patients of type 2 DM and 30 age and sex matched healthy individuals as control group. Inclusion criteria: Type 2 DM without any complications, aged between 30 to 60 years, males and females. Exclusion criteria: Diabetes mellitus other than type 2, history of smoking, alcoholism and Pregnant women. Blood sample was collected in fluoride bulb (fasting and post prandial) for glucose estimation and in plain bulb for other parameters. Blood glucose was estimated by glucose oxidase peroxidase (GOD-POD) enzymatic method. Serum protein carbonyl estimated

by dinitrophenyl hydrazine (DNPH) method<sup>7</sup>, total nitrite (nitrate and nitrite) concentrations by Griess reaction<sup>8</sup> and Malondialdehyde (MDA) by thiobarbituric acid (TBA) method<sup>9</sup>. Statistical Analysis: Data is expressed as Mean SD. Comparison of various biomarkers of oxidative stress between diabetes and normal group was done by applying ' Z' test. The difference was said to be significant when P was < 0. 001 and highly significant when P < 0. 0001. Statistical correlations between different biomarkers of oxidative stress in diabetic patient were expressed as Pearson's correlation, moderate correlation was said when ' r' value = or > 0. 5.

## Results:

Blood glucose estimation was done as baseline investigation (Table 1). Blood glucose was significantly higher in diabetic patients as compared to control group.

### Table 1- Blood glucose level in diabetic and control group.

Control (n = 30)	Mean	S. D.	Diabetic Patients (n = 60)	Mean	S. D.
Blood glucose level(fasting) (mg/dl)	90. 63	7. 03	140. 4	50. 41	
Blood glucose level(Post-prandial) (mg/dl)	121. 26	10. 76	202. 32	65. 25	

Table 2 shows the levels of various biomarkers of oxidative stress in type 2 DM and control group.

### Table 2- Serum levels of protein carbonyl, nitric oxide and MDA in control and diabetic patients.

Sr. No.	Biochemical Parameter	Control group (Mean	SD)	Diabetic Patients (Mean	SD)	Significance test
1	Protein Carbonyl (CO) (nmol/mg protein)	1. 51	0. 22	3. 28	0. 75	Z= 17. 7*
2	Total nitrites (NOx) (μmol/L)	46. 6				

17. 5176. 1 68. 12Z= 13. 84\*3MDA(nmol/ml)3. 12 0. 778. 3 1. 19Z= 24. 67\*\* P < 0. 0001, highly significantThe mean protein carbonyl level (nmol/mg protein) of type 2 diabetic patient was significantly higher (3. 28 0. 75) than that of control group (1. 51 0. 22), (P < 0. 0001). The mean nitric oxide level (in terms of NOx) ( $\mu$ mol/L) of diabetic patients was statistically higher (176. 1 68. 12) than that of control group (46. 6 17. 5), (P < 0. 0001). The mean MDA level (nmol/ml) of diabetic patients was statistically higher (8. 3 1. 19) than that of control group (3. 12 0. 77), P < 0. 0001. All biomarkers of oxidative stress in type 2 DM were statistically higher (P < 0. 0001) than that of control group.

### **Table 3- Correlation between protein carbonyl, NOx and MDA in diabetic patients.**

Sr. No. Biochemical parameter(' r' value)1)Protein carbonyl and Nox-0. 076

\*2)Protein carbonyl and MDA0. 5 \*\*3)NOx and MDA-0. 086\*\*No correlation,

\*\*Moderate correlation. Statistical correlations between biomarkers of oxidative stress in diabetic patient were shown that there was no correlation between protein carbonyl and NOx, No correlation between NOx and MDA.

There was moderate correlation between protein carbonyl and MDA (' r' value = 0. 5).

### **Discussion:**

Diabetes is characterized by high glucose concentration that leads, via several mechanisms, to an increased production of reactive oxygen species (ROS). Free radicals are constantly being produced in the body, as a result of normal metabolic processes. Under physiological conditions damage due to

free radicals is countered by antioxidants. Sometimes, excessive free radical formation occurs in the body, and the antioxidant system in the body cannot cope with the situation, results into oxidative stress<sup>2, 3</sup>. Several mechanisms seen to be involved in the genesis of oxidative stress in diabetes mellitus, namely, glucose auto-oxidation, protein glycation, advanced glycation end products (AGE) formation<sup>10</sup>, Sorbitol system activation and functional limitation of HMP shunt, leading to decrease in glutathione synthesis<sup>11</sup>. Non-enzymatic glycation is a process by which glucose is chemically bound to amino groups of proteins, but without the help of enzymes. It is classical covalent reaction in which, by means of N-glycosidic bonding, sugar protein complex is formed<sup>12</sup>. Glycated protein undergoes irreversible modification leading to Maillard products or AGEs. As glycated proteins, AGEs are also able to produce oxygenated free radicals<sup>12</sup>. Glucose at high concentration is preferentially metabolized via the polyol pathway. Therefore, an enhancement of the polyol pathway results in an intracellular depletion of NADPH. This negatively influences other enzymes and systems involved in defensive processes against oxidative stress<sup>11</sup>. In our study, there was increased level of protein carbonyl (CO) group in type 2 diabetic patients compared to control subjects. Reactive oxygen species (ROS) rapidly inactivate NO leading to the formation of peroxynitrite (ONOO<sup>-</sup>). Peroxynitrite is a toxic oxidant capable of damaging many biological molecules. Reduced NO bioavailability possibly contribute to the development of insulin resistance<sup>13</sup>. The resulting oxidative stress can play key role in pathogenesis of diabetes. Superoxide radicals generated by the reduced form of NADPH oxidase may thus contribute to impaired endothelium-

dependent vascular relaxation by the inactivation of nitric oxide<sup>14</sup>.

Endothelial dysfunction can be described as endocrine disorder, and it is genetic or acquired. In both conditions it is likely that the increased endothelial generation of superoxide anion can lead to rapid inactivation of NO and, therefore, exacerbate endothelial injury. Endothelial dysfunction is characteristic of several diseases like diabetes, atherosclerosis, angina etc.

15. In particular, an increased oxidative stress seems to be the main mechanism through which insulin resistance causes endothelial dysfunction<sup>16</sup>. We observed increased level of NO<sub>x</sub> in type 2 DM compared to normal subjects. NO<sub>2</sub> (nitrite) levels by Griess reaction may reflect accurately the endogenous synthesis of NO from I-NOS<sup>8</sup>. Moshage H, et al<sup>17</sup> evaluated nitrate and nitrite determination in plasma. According to them plasma nitrate and nitrite determinations are increasingly being used in clinical chemistry as marker of the activity of nitric oxide synthase and the production of nitric oxide radicals. We found increased level of MDA in type 2 diabetic patients compared to normal subjects. The development of diabetic complications in diabetes is closely related to increased generation of superoxide anion and decreased nitric oxide (NO)<sup>18</sup>. Piconi L, et al<sup>19</sup> suggested that there is close link between hyperglycemia, oxidative stress and diabetic complications. Limitation of our study: small sample size, duration of disease, correlation between degree and /or duration of oxidative stress in type 2 DM and development of diabetic complications is not studied. Further study is needed to study markers of oxidative stress in DM with and without complications, its correlation with duration of disease,



glycemic control, different complications of DM and what is effect of antioxidant supplementation in DM treatment.

### **Conclusion:**

Biomarkers of oxidative stress such as protein carbonyl, NO and MDA are increased in type 2 DM. There is increased lipid peroxidation, insulin resistance, reduced bioavailability of endothelial nitric oxide (NO) and endothelial dysfunction. These effects of oxidative stress may play important role in development of complications of DM. This suggests that there is increased oxidative stress in type 2 DM and antioxidant supplementation along with anti-diabetic therapy may be beneficial in preventing or postponement of complications of diabetes mellitus.