

# Oxidative Stress and Antioxidant Levels in Diabetes Mellitus Patients

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## ABSTRACT

High glucose level in diabetic mellitus can lead to free radical production causing oxidative stress. The present study has been undertaken to study the oxidative stress by measuring the antioxidant levels in diabetes mellitus patients. The level of malondialdehyde (MDA), ischemia modified albumin (IMA), ascorbic acid and vitamin E concentration in serum of normal and diabetic subjects of Chhattisgarh has been studied. A total of 125 diabetic and normal healthy subjects both male and female ranging in the age group 30-65 years were included in this study. The concentration of vitamin E, MDA, IMA and ascorbate in the serum of diabetic subjects was quantitatively determined and compared with that of normal control subjects. Student's *t* test was used to evaluate the significant of differences between the parameters measured. The concentration of MDA and IMA increased significantly in diabetic patients as compared to normal control subjects. The ascorbate content of diabetic subjects significantly declined as compared to that of control subjects. Also, the level of the vitamin E declined about 40-50% in diabetic subjects of both the sexes compared to control subjects. The increased level of oxidative stress biomarkers such as MDA and IMA in diabetic patients accompanied with decreased level of non enzymatic antioxidants such ascorbic acid and vitamin E as observed in the present work could cause complications in patients leading to oxidative damage to proteins, lipid and nucleic acids.

**Keywords:** antioxidants, diabetes mellitus, oxidative stress, malondialdehyde, ascorbic acid.

## INTRODUCTION

Diabetes mellitus (DM) represents a group of chronic diseases characterized by high levels of glucose in the blood that results from defects in insulin production, insulin action, or both. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome. <sup>[1]</sup> Patients with diabetes show an increased incidence of

atherosclerotic cardiovascular, peripheral arterial, cerebrovascular disease, hypertension and abnormalities of lipoprotein metabolism. <sup>[2]</sup> As per estimate of the International Diabetes Federation (IDF), the total number of people in India with diabetes which was around 50.8 million in 2010 would be 87.0 million by 2030. <sup>[3]</sup>

Oxidative stress plays a major role in cellular injury from hyperglycemia. High glucose level can lead to free radical production. Weak defence 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 of oxidative stress. [4] Oxidative stress is defined as a state in which oxidation exceeds the capacity of antioxidant systems in the body secondary to a loss of the balance between them. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. [5] Studies have demonstrated that hyperglycemia-induced oxidative stress led to the activation of mitogen-activated protein kinase (MAPK), which may have contributed to neuronal pathogenesis. [6, 7]

Increased oxidative stress as well as reduction in antioxidant capacity could be related to the complications in patients with diabetes such as oxidative DNA damage and insulin resistance. Due to decrease in antioxidant potential of plasma, complications of diabetes increase which include cardiovascular disease, nerve damage, blindness, and nephropathy. Thus, the increasing incidence of diabetes is a significant health concern beyond the disease itself. [8]

Oxidative stress alters major biomolecules in the cell and status of plasma antioxidant potential during Type2 DM. These include lipid peroxidation, protein oxidation, glutathione level, superoxide dismutase and catalase effects. Out of all these processes, lipid peroxidation is one of the highly toxic mechanisms of generating ROS. Assessment of the extent of oxidative stress using biomarkers is interesting from a clinical standpoint. The markers found in blood, urine, and other biological fluids may provide information of diagnostic value. [9]

Taking into consideration the above points, the present study has been undertaken to determine the oxidative stress by measuring the antioxidant levels in diabetes mellitus patients. The level malondialdehyde (MDA), ischemia modified albumin (IMA), ascorbic acid and vitamin E concentration in serum of normal

and diabetic subjects of Chhattisgarh has been studied.

## **MATERIALS AND METHODS**

### **Place of study**

The present study was carried out in Department of Biochemistry, Pt. J.N.M. Medical College, Raipur and approved by the ethical committee. The purpose of study was clearly explained to every patient before enrolling them.

### **Study population**

A total of 65 patients who were diagnosed for DM 2 reporting to Dr. B.R Ambedkar Hospital for their regular blood sugar tests were recruited for the study. They had post prandial blood glucose levels of more than 140mg/dL. Another 60 volunteers were recruited from healthy blood donors and the ones who came for medical fitness tests. Pregnant ladies, cancer patients and patients taking antioxidant supplements were excluded from the study.

### **The patients**

The study subjects included both male and female sexes of various age groups ranging from 30 to 65 years age group. In total an average of 125 patients were studied. The personal information, physical characteristics, habits and other relevant information of the patients were obtained.

### **Collection of samples**

Blood sample was collected by expert technicians of clinical biochemistry sample collection laboratory of Dr. B. R. Ambedkar Memorial Hospital (BRAMH), 5.0 ml of venous blood was drawn in vacutainers and allowed to clot at room temperature for 20 min, centrifuged at 3000 rpm for 10 minutes and the sera was then divided into aliquots and stored frozen at (-20° C) for determination of vitamin E and lipid peroxidation product MDA and IMA. The rest of the blood was collected in EDTA vacutainer, centrifuged and plasma

was kept in aliquots at -20° C for determination of ascorbic acid.

**Biochemical estimation**

Lipid peroxidation product MDA was estimated by method of Cynamon et al. [10] Vitamin C was estimated by 2, 4 dinitro phenyl hydrazine (DNPH) method according to Omaye et al. [11] The albumin Cobalt-Binding Test was used to detect IMA. [12] Vitamin E determination was done by the method based on the principle of reduction of ferric to ferrous ions, which can then form a red complex with  $\alpha$ - $\alpha'$  dipyritydyl. [13]

**Statistical analysis**

The data presented in this study is the mean of three independent measurements taken at different times. The standard deviation (SD) was calculated using MS Excel and the data were presented as mean  $\pm$  SD. Student's *t* test was used to evaluate the significant of differences between the parameters measured.

**RESULTS**

The physical characteristics of healthy control and diabetic subjects have been shown in table 1. Out of total 125 subjects 65 were diabetic and 60 were normal age and sex matched controls Overall the study group comprised of 34 females (52%), 31 males (47%) and the control group comprises of 35 (58.33%) females and 25 (41.66%) males. The participants were between 30 and 65 years with a mean of 45.75 years of age. The mean BMI of control and diabetic subjects were measured as 24.89 and 27.81 Kg/m<sup>2</sup> respectively.

**Table 1: Physical characteristics of control and diabetic subjects.**

Characteristics/parameters	Control	Case
	Mean ( $\pm$ SD)	Mean ( $\pm$ SD)
Average age (years)	46.21 ( $\pm$ 2.32)	52.6 ( $\pm$ 5.29)
Height (m)	1.59 ( $\pm$ 0.93)	1.59 ( $\pm$ 0.025)
Weight (Kg)	62.49 ( $\pm$ 3.68)	70.45 ( $\pm$ 6.25)
BMI (Kg/m <sup>2</sup> )	24.89 ( $\pm$ 3.4)	27.81 ( $\pm$ 5.21)

The mean serum MDA concentration of control male and female subjects was determined as 202.64 and 217.48 nmol/100 ml where as in case of the male and female belonging to DM 2 individuals the mean MDA content were 281.049 and 266.820 nmol/100ml respectively. An increase of 39% in MDA content of diabetic male was observed as compared to the control males where as in diabetic female the increase was only 22% as compared to the control females.

The mean IMA content of males and females of control subjects was 0.42 and 0.34 absorbance units; while the mean concentrations of IMA in diabetic male and female subjects were calculate to be 0.61 and 0.46 absorbance units respectively. The IMA content of both male and diabetic subjects increased significantly compared to that of control subjects of respective sex. IMA content in male diabetic subjects increased by 45% where as in female subjects the IMA content increased about 35% over the control females.

The mean ascorbic acid content of control male and female subjects were 3.2 and 2.8 mg/dl respectively where as ascorbate content of diabetic male and female subjects noticed to be 0.84 and 0.911 mg/dl respectively. The ascorbate content of both male and female diabetic subjects significantly declined as compared to that of control male and female subjects.

Vitamin E concentration in control male and female subjects was estimated as 13.87 and 15.51 mg/l respectively. In diabetic male and female subjects the content of Vitamin E was measured as 9.27 and 11.09 mg/l. The level of the vitamin E declined about 40-50% in diabetic subjects of both the sexes compared to control subjects.

The content of MDA, IMA, ascorbic acid and Vitamin E in control and diabetic subjects has been summarised in table 2.

**Table 2: MDA and IMA, ascorbaic acid and vitamin E content in male and female subjects of control and diabetic subjects.**

Parameters	Control		Case		P value	
	Male Mean (±SD)	Female Mean (±SD)	Male Mean (±SD)	Female Mean (±SD)	Male	Female
Postprandial sugar (mg/dl)	112.52 (±4.89)	110.25 (±4.05)	246.87 (±8.52)	224.27 (±7.56)	-	-
MDA (nmol/100 ml)	202.64 (±13.21)	217.48 (±10.54)	281.049 (±7.26)	266.820 (±8.46)	<0.005	<0.005
IMA (absorbance units)	0.42 (±0.013)	0.34 (±0.021)	0.61 (±0.09)	0.46 (±0.021)	<0.05	<0.005
Ascorbic acid (mg/dl)	3.20 (±0.241)	2.80 (±0.316)	0.84 (±0.024)	0.911 (0.018)	<0.005	<0.05
Vitamin E (mg/l)	13.87 (±2.05)	15.51 (±4.36)	9.27 (±2.12)	11.09 (±3.14)	<0.05	<0.05

\*P' value <0.05 indicates significance difference between case and control.

## DISCUSSION

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Hyperglycaemic condition is known to generate ROS, which in turn cause damage to the cells in many ways that leads to secondary complications in diabetes mellitus. [14] The lipid peroxidation product, MDA has been recognized as a primary biomarker of free radical mediated lipid damage and oxidative stress. [15] In the present study, the MDA levels have been measured in diabetic and healthy control subjects. Higher level of MDA in diabetic subjects observed in this study could be attributed to higher ROS.

The study shows that, there is significant elevation in MDA concentration of diabetic subjects compared to that of control healthy individuals. Increased MDA level in plasma, serum, and many others tissues have been reported in diabetic patients. [16] Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and non-enzymatic antioxidants. [17] In the present study, we have observed significant decreases in the activity of non-enzymatic antioxidants vitamin E and ascorbic acid in diabetic subjects as compared with controls. Vitamin E acts as lipid peroxidation chain breaking antioxidant as well as quenches the free radicals. Vitamin C reacts directly with superoxide and hydroxyl ions and acts

synergistically with vitamin E as chain breaking antioxidant by regenerating the reduced form of vitamin E from tocoperoxy radical.

Clinical studies have reported that significantly higher lipid peroxidation is associated with high glucose levels as observed by the fasting glucose and HbA1c levels. [16] The plasma antioxidant level is significantly lower in diabetic subjects with poor glycaemic control than healthy subjects, while patients with good glycaemic control had plasma antioxidative values similar to controls. Another study has reported significant reduction in biological antioxidant potential in sciatic nerve homogenates of diabetic animals. [18] Thus, oxidative stress event in diabetics coexists with a decrease in the antioxidant status that also has been observed in the present work could further increase the deleterious effect of free radicals in type 2 DM patients.

Ischemia-modified albumin (IMA) is a promising biomarker for evaluating patients with ischemic events. Some previous studies have reported that compared to healthy controls, patients with type 2 diabetes and chronic microvascular complications have higher serum levels of IMA. [19] One of the major findings of the present study is significant increase in serum IMA content of both male and female type 2 patients. Some studies have reported that compared to healthy controls, patients with type 2 diabetes and chronic microvascular complications have higher serum levels of IMA. [20] The findings of the present study further substantiate IMA as a

biomarker of ischemic associated diseases including diabetics.

The increased level of oxidative stress biomarkers such as MDA and IMA in diabetic patients accompanied with decreased level of non enzymatic antioxidants such ascorbic acid and vitamin E as observed in the present work could cause complications in patients leading to oxidative damage to proteins, lipid and nucleic acids. This may also cause insulin resistance in type 2 diabetic patients. [21] This may further lead to more complications in diabetes like cardiovascular diseases, nerve damage, and blindness etc.

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