ISSN: 2394-1391

Original Article

Indian Journal of Biology
Volume 2 Number 2, July - December 2015
DOI: https://dx.doi.org/10.21088/ijb.2394.1391.2215.3

Oxidative Stress in Diabetic Patients

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Abstract

Oxidative stress plays a major role of free radicals generation disproportionately in diabetes mellitus by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, nitric oxide level and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including catalase, glutathione peroxidase, lipid peroxidation, nitrite concentration and their consequences, are discussed in this research article. Biochemical studies were carried out in 10 diabetes patient whose age range from 45-55 years. For control data, 10 individuals in the same group (45-55 years), socio-economic status and who were not suffering diabetes mellitus as a control groups.

Keywords: Oxidative Stress; MDA; GPx; SOD; Free Radicals.

Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy [1]. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, Diabetes, Heart Failure, Myocardial Infarction, Alzheimer's disease and chronic fatigue syndrome, but short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis. Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling [2,3]. In modern medicine, regular physical exercise is an important tool in the prevention and treatment of diseases including diabetes. Although acute exhaustive exercise increases oxidative stress, exercise training has been shown to up regulate antioxidant protection. This highly reactive and short-living class of molecules is known as reactive oxygen species (ROS). ROS production is a side effect of the normal metabolism of the cell, which developed a series of enzymes able to disarm them. Superoxide dismutase, catalase, and glutathione peroxidase are powerful weapons that act together with antioxidant molecules introduced with diet to protect the organism. In healthy subjects there is a balance between ROS formation and elimination [4-5]. Every time this balance is lost due to an augmented production of reactive species or due to a reduction in antioxidant production or activity there is a condition of oxidative stress.

The foregoing research indicated the effect of free radicals generated in diabetic patients due to oxidative stress which involves the estimation of activity of antioxidant enzymes and the measurement of levels of nitric oxide and lipid peroxidation. The

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antioxidant enzymes includes Catalase and Glutathione peroxidase to be estimated in the blood of Diabetic patients and the level of nitric oxide and lipidperoxidation to be estimated by spectrophotometry analysis [6-7].

Materials and Methods

Biochemical studies were carried out in 10 diabetes patient whose age range from 45-55 years. For control data, 10 individuals in the same group (45-55 years), socio-economic status and who were not suffering diabetes mellitus as a control groups. 4ml of heparinized whole blood samples were collected from Gunupur hospital and clinics at Gunupur.

Nitrate and nitrate concentrations in plasma were determined by using Griess reaction in which NO_2 reacts with 3% sulfanilamide in 0.3% Napthalene-ethylene diamine dihydrochloride, forming chromophore [8-9].

The extent of lipid peroxidation in biological sample is estimated by the thiobarbutiric acid test (TBA test). In this the amount of malondialdehyde (MDA) formed in the samples is taken as the index for the extent of lipid peroxidation. MDA is a highly reactive three-carbon dialdehyde produced from lipid hydroperoxides. It is measured by the TBA test [10,11].

Catalase a 24.5 kDa molecular weight, antioxidant enzyme contains heme group bound to its active site. It catalyzes the conversion of high concentrations of hydrogen peroxides formed by the dismutation of SOD and oxygen. This activity measured spectrophotometrically at 240 nm. Azide or cyanide inhibits catalase [12].

Activity of Glutathione peroxidase was measured by the method of Paglia and Valentine [13]. In this method, reduced glutathione(GSH) is converted to oxidized glutathione (GSSG) by glutathione peroxidase which measured spectrophotometrically at 340nm.

Results

The result showed a significant increase in the level NO and lipid peroxidation and the activities of antioxidants includes GPx and Catalase were decreased significantly in diabetic patient's blood when compared to control groups.

Nitric Oxide Levels in Plasma

Measurement of nitrite in plasma is the indicative of the amount of nitric oxide. The nitrite levels were observed to be increased significantly ($P < 0.001$) in diabetes patient as compared to the values in control shown in Fig. 1 and Table 1.

Lipid Peroxides Levels

The lipid peroxidation products expressed as MDA equivalents observed significantly ($P < 0.001$) as compared to the values in control shown in Fig. 2 and Table 2. High levels of MDA in diabetic patient were indicative of increase oxidative stress.

Catalase Activity

Catalase activity decreased significantly ($P < 0.001$) in Diabetic patients when compare to control shown in Fig. 3 and Table 3.

Glutathione Peroxidase Activity

GPx activity decreased significantly ($P < 0.001$) as compared to the vales in control shown in Fig. 4 and Table 4.

Table 1: Nitric oxide (nitrite) levels in plasma values are expressed as mg of nitrite/ml of plasma

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.725 ± 0.06
P	< 0.001	
Diabetic	10	1.79 ± 0.08

Table 2: Lipidperoxidation (MDA) levels in plasma. Values are nanomoles of MDA/ml of plasma

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.116 ± 0.005
P	< 0.001	
Diabetic	10	1.036 ± 0.052

Table 3: Catalase Activity in Plasma values are expressed in mg/ml

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.264 ± 0.008
P	< 0.001	
Diabetic	10	0.086 ± 0.004

Table 4: GPx Activity in plasma values are u moles NADPH oxidized/min/mg protein

Experimental Condition	No. of samples	Mean ± SEM
Control	10	0.214 ± 0.004
P	< 0.001	
Diabetic	10	0.091 ± 0.003

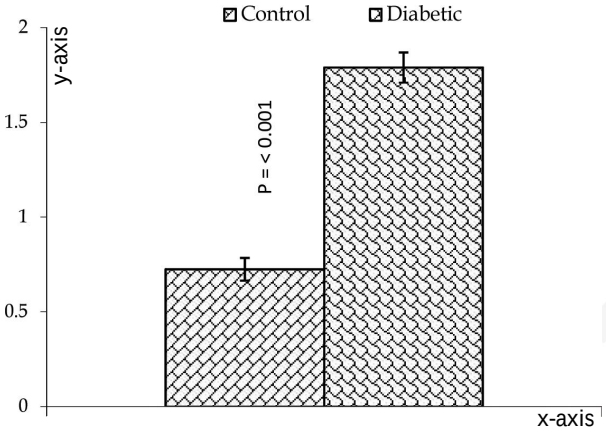

Fig. 1: Nitric oxide (nitrite) levels in plasma values are expressed as mg of nitrite/ml of plasma

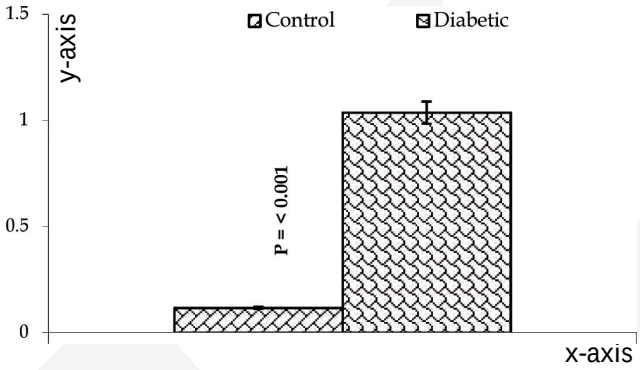

Fig. 2: Lipidperoxidation (MDA) levels in plasma values are nanomoles of MDA/ml of plasma

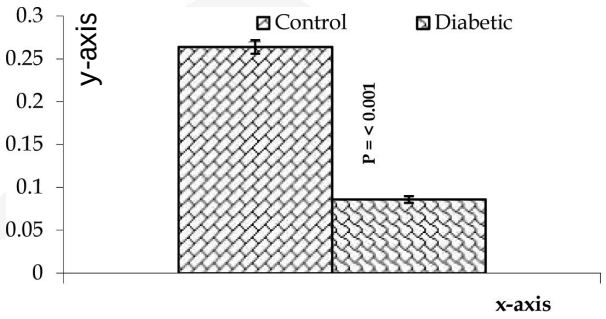

Fig. 3: Catalase Activity in Plasma. Values are expressed in mg/ml

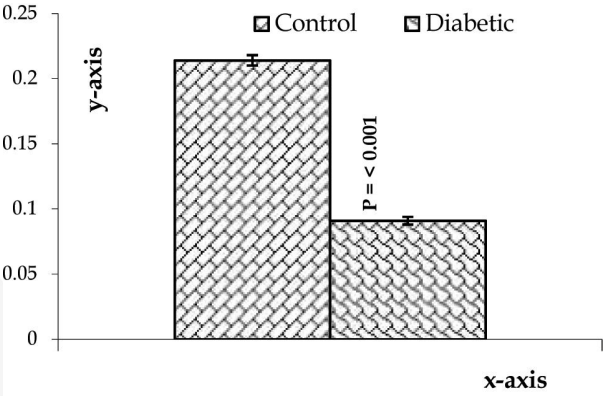

Fig. 4: GPx Activity in plasma values are u moles NADPH oxidized/min/mg protein

Discussion

In this study, we reported the frequency of catalase deficiency which observed among the patients with low activity and the blood catalase activity of randomly selected diabetic patients [14,15]. Increased in lipid peroxidation in diabetes mellitus is due to excess formation of free radicals. Glycosylated protein, auto oxidation, reduced superoxide dismutase enzyme and ascorbic acid and lack of reduced glutathione are other causes for oxidative stress. Here all groups of diabetes mellitus shows statistically significant increase in serum lipid Peroxide levels. In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides and nitric oxide in plasma may be due to the abnormal lipid metabolism and by oxidation of amino acids. Maximum increase in lipid peroxide was found in group of diabetes mellitus with complication [8,16,17]. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [18]. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid per oxidation and tissue damage in diabetes [19]. The glycated protein might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid per oxidation in diabetes mellitus [12,20]. A deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to higher concentration of peroxide. There may be imbalance between

production and scavenging of free radical produced due to the lack of antioxidant system [21,22]. Peroxidation of apolipoproteins may affect the lipoprotein metabolism. It is suggested that apo- A has an antioxidant effect, but due to the peroxidation the antioxidant property of apo-A is lost. Higher levels of lipid peroxides were observed in diabetic subject with vascular complication. This increase in lipid peroxide may be due to the increased activity of the free radical formation [23]. It has been suggested that the increase in triglyceride may be due to insulin deficiency which results faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue resulting in hyperglycemia [24]. High level of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may be due to the obesity, increase calorie intake and lack of muscular exercise in the patients of diabetes mellitus [25].

Conclusion

In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen-free radicals through glucose autoxidation and nonenzymatic glycation. The antioxidant capacity is always decreased in diabetic patients, but it seems necessary to measure all the components to ascertain the reasons. The activities of Catalase and GPx were significantly low in diabetic patients where as the level of nitric oxide and lipid peroxidation were significantly increases. The enzyme activities in diabetic patients are lower than that of control, but the differences are more significant. It seems that the reduction in levels of other antioxidant enzymes and substances are involved in the decreased antioxidant capacity in diabetic patients. In view of low activities of catalase and GPx in patient's supplementary trace elements such as Selenium, Copper, Zinc and Manganese, the essential components of the enzymes structures may be useful in prevention of oxidative stress.

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