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Evaluation of Oxidative Stress and Antioxidant Status in Relation to Glycemic Control in Type 1 and Type 2 Diabetes Mellitus Patients

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ABSTRACT

Oxidative stress and antioxidant status was evaluated in relation to glycemic control in Type 1 and 2 Diabetes Mellitus patients (DM). A total of 69 DM patients (34 T1 and 35 T2DM cases) were enrolled in the study along with 15 healthy subjects. The patients were grouped into those with good glycemic control and others with poor glycemic control. Serum fructosamine levels were also determined as a supportive parameter for confirming glycemic status. Serum Malondialdehyde (MDA) level was used as a marker of oxidative stress and serum catalase activity was quantitated for an assessment of anti-oxidant status of the patients. Mean MDA levels in poor glycemic control group of T1 and T2DM were significantly higher ($p < 0.01$) not only than the means of the healthy controls but also than the means of diabeteic groups with good glycemic control ($p < 0.01$). Mean serum catalase activity was significantly reduced in DM patients of all the groups compared to that of healthy controls ($p < 0.05$) indicating considerable reduction in the anti-oxidant status of the patients.

Key words: Diabetes mellitus, oxidative stress, reactive oxygen species, MDA, catalase

INTRODUCTION

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are believed to play a dual role as both deleterious and beneficial species (Valko *et al.*, 2007). Normally both these species are generated by well regulated enzymes such as NO Synthase (NOS) and NAD(P)H oxidase isoforms. However excessive production of ROS (arising either from mitochondrial electron transport chain or excessive stimulation of NAD(P)H oxidase) results in oxidative stress a process in which Superoxide radicals interact with lipids, proteins and DNA (Bains and Shaw, 1997). On the contrary the beneficial effects of ROS/RNS are observed at low/moderate concentrations for example in defense against infectious agents as observed in polymorphonuclear phagocytosis (Nagl *et al.*, 2002). Reports are available in the literature that claim that excess ROS within cells are involved in intracellular signaling which induce and maintain oncogenic phenotype of cancer cells; however, they can also induce cellular senescence and apoptosis and hence, can fuction as anti-tumorigenic species (Valko *et al.*, 2007). Oxidative stress has been function as implicated as

an important pathophysiological process to linked to common complex human diseases like Diabetes mellitus (Yorek, 2003) and Cardiovascular diseases, hypertension, atherosclerosis, cancer, neurodegenerative disease, rheumatoid arthritis and aging (Bains and Shaw, 1997).

The aim of the present study is to evaluate oxidative stress in relation to glycemic controls in diabetes mellitus. The importance of such studies can be realized in both (T1 and T2DM) due to high prevalence of T2DM in the last two decades and it is estimated that the number of T2 diabetes across the world is likely to be more than double (Chaturvedi, 2007). High incidence of cardiovascular disease has been observed in diabetic patients which is associated with high morbidity and mortality (Brownlee, 2001). However, microvascular complications such as retinopathies, nephropathy and neuropathy are also frequently observed in patients with diabetes (both T1 and T2) (Brownlee, 2001; Ceriello, 2011; Yorek, 2003). Oxidative stress is considered not only as an important mechanism contributing not only to the causation of diabetes mellitus but also to its associated complications (Ceriello, 2011). Various lines of evidence suggest that hyperglycemia observed in DM cases induces excessive generation of free radicals. Hence, in the present study we evaluated Oxidative stress in both T1 and T2DM patients in relation glycemic control by measuring serum MDA levels, a marker of Oxidative stress. Fasting blood glucose and serum fructosamine levels as indicators of extent of glycemic control. Serum catalase activity has been determined as a parameter of anti-oxidant status. An important aspect of this study is that it reports not only MDA levels as an indicator of extent of lipid peroxidation but also serum catalase activity as a determinant of anti-oxidative status of the DM patients.

MATERIALS AND METHODS

This study was conducted on the patients diagnosed as suffering from DM (T1/T2DM) based on the provisional diagnosis and Fasting Plasma Glucose (FPG) levels higher than 126 mg dL^{-1} (ADA, 2013). These cases were visiting the Diabetic clinic-out patient wing of Department of Medicine, Princess Esra Hospital, Hyderabad, India.

The study protocol was approved by Institutional Ethics Committee. An informed consent was obtained from all the patients and controls participating in the study. The control group (Group 1) comprised of 15 healthy subjects both males and females in the age group of 25-50 years with no history of hypertension and were non smokers. The T1DM group (Group 2) comprised of 34 patients (19 females and 15 males) falling in the age range of 13-33 years. Estimation of serum creatinine levels, examination of deep and superficial reflexes, touch, position and vibration sense were performed to rule out any complications. X-ray chest, ECG was taken to rule out any chronic infection and ischemic heart disease. Group 3 comprised of 35 T2DM cases (19 males and 16 females) falling in the range of 31-52 years. All the investigations carried out to rule out any complications and chronic infections in T1DM cases were also done in T2DM patients to rule out any complications. The T1 and T2DM cases were further subgrouped into those with poor glycemic control and good glycemic control (T1DM Group 2a: Poor glycemic control (FPG<126 mg dL^{-1}) and Group 2b: Good glycemic control (FPG>125 mg dL^{-1}); Similarly, 3a and 3b for T2DM).

Collection of sample: Fasting venous blood samples (5 mL) were collected from both the patients and healthy subjects and were distributed into 2 vials. One with anti-coagulant and other without anticoagulant to collect the serum which was used to estimate malonaldehyde, catalase and fructosamine.

Estimation of biochemical parameters: Fasting Plasma Glucose levels (FPG) were determined using the blood glucose oxidase method. Serum fructosamine levels were determined according to modified Winzlers procedure as described by Kennedy *et al.* (1979) and the values were expressed as mg percent. Serum MDA levels were determined by Thiobarbituric Acid Substances (TBARS) assay (Esterbauer and Cheeseman, 1990) where one molecule of MDA in serum reacts with two molecules of TBA in acidic medium and gives rise to a pink color complex which is measured spectrophotometrically at 532 nm against distilled water blank. The values were expressed as nanomoles per deciliters (nmoles dL^{-1}). Serum catalase activity was determined according to the method of Goth (1991). The method was based on spectrophotometric assay of Hydrogen peroxide based on the formation of stable color complex with Ammonium Molybdate. The enzyme activity was expressed as $kU\ L^{-1}$.

RESULTS

Details of the mean levels of plasma glucose, serum fructosamine, MDA and catalase activity are given in the Table 1. Levels of fasting plasma glucose and serum fructosamine have been determined as indicators of glycemic control and to subgroup the T1 and T2DM cases into those with good and poor glycemic control. All the individuals in the healthy subjects (Group 1) had FPG <100 mg dL^-: While T1DM patients and T2DM patients with good glycemic controls had FPG levels less than the cut-off the value <125 mg dL^{-1} as recommended by ADA (2013). Similarly the mean serum fructosamine levels did not differ significantly in these groups (Group 1, 2b and 3b) indicating good glycemic control in these patients. In T1DM and T2DM patients with poor glycemic control the mean FPG values were higher than 126 mg dL^{-1} (a value recommended by ADA to diagnosing a case suffering from diabetes mellitus).

Serum fructosamine levels were significantly ($p<0.01$) higher in T1 and T2DM patients with poor glycemic controls (Group 2a and 3a) when compared with healthy subjects and also patients with good glycemic controls. However, the mean fructosamine levels in diabetic patients with good glycemic controls (Group 2b and 3b) and in healthy subjects did not differ significantly.

Statistically significant difference was noted between the mean levels of fructosamine in T1DM patients with good and poor glycemic controls ($p<0.01$). Similar results were obtained in T2DM cases with poor and good glycemic controls ($p<0.01$).

Serum MDA level were significantly higher ($p<0.001$) in diabetic patients with good and poor glycemic controls (Group 2a, 2b and 3a, 3b) when compared with healthy subjects (Group 1).

Table 1: Levels of fasting plasma glucose, serum fructosamine, malonaldehyde and catalase activity in patients and controls

Groups	No.	Fasting blood glucose (mg dL^{-1})	Serum fructosamine (mg %)	Malonaldehyde (nmoles dL^{-1})	Catalase activity (kU L^{-1})
1 (Control: Healthy subjects)	15	78.40±8.70	147.40±24.60	176.50±18.03	44.60±6.90**
2a (T1DM poor glycemic control)	16	221.12±90.01**	244.68±68.88**	550.00±133.76***	26.81±11.70
2b (T1DM good glycemic control)	18	74.20±15.09	152.22±20.90	417.72±123.67	33.05±12.37*
3a (T2DM poor glycemic control)	16	192.31±88.69**	236.87±78.60**	382.75±67.40**	25.75±4.00**
3b (T2DM good glycemic control)	19	88.52±15.37	149.21±27.80	277.05±50.35	30.63±4.83

*$p<0.05$, **$p<0.01$, ***$p<0.001$. Significant level for (A) Serum MDA levels, (1): Group 3a vs. Group 3b $p<0.05$, (2): Group 2a vs. Group 2b $p<0.05$, (B) Serum Catalase activity, (1): Group 1 vs. Group 2a $p<0.01$, (2): Group 1 vs. Group 2b $p<0.05$, (3): Group 1 vs. Group 3a $p<0.01$, (4): Group 1 vs Group 3b $p<0.05$

Between subgroups Group 2a T1DM patients with poor glycemic control showed significantly higher ($p<0.05$) serum MDA levels compared to Group 2b patients with good glycemic control. Similar results were obtained when Group 3a and 3b of T2DM cases were compared.

In this study, serum catalase levels were significantly lower in diabetic patients of all groups (Group 2a, 2b, 3a and 3b) when compared with healthy subjects (Group 1) ($p<0.05$). There was no significant difference in catalase activity in T1DM patients (Group 2a) and those with controlled blood sugar levels (Group 2b). However, the activity of this enzyme in Group 3a (poor glycemic control) of T2DM was significantly lower than in Group 3b (T2DM patients with good glycemic controls) ($p<0.05$).

DISCUSSION AND CONCLUSION

Quantitative estimation of MDA, an end product of lipid peroxidation produced by interaction of free radical with membrane phospholipids, is considered as a reliable indicator of oxidative stress. There are reports implicating the role of hyperglycemia in increasing free radical generation and consequent oxidative stress induced damage to cells and tissues in both type T1 and T2DM cases resulting in the complications associated with DM (Johansen *et al.*, 2005; Moussa, 2008; Giugliano *et al.*, 1996). Significantly elevated levels of MDA in type T1 and T2DM patients with poor glycemic controls (Group 2a and 3a) is attributed to chronic hyperglycemic state. Hyperglycemia has been implicated in reactive oxygen (ROS) production. It has been reported that the elevated plasma glucose levels results in increase in production of electron donors ($NADH/H^+$) from TCA cycle in tissues like retina, lens, vascular endothelial cells, smooth muscle cells and pancreatic beta cells which do not require insulin for uptake of glucose. This increased production of electron donors results in the transfer of single electrons (instead of usual electron pairs) to oxygen producing superoxide radicals and other reactive oxygen species (instead of water as the usual end product) (Ceriello, 2011).

The superoxide radical so produced is reported to partly inhibit the activity of key glycolytic enzyme, Glyceraldehyde-3-phosphaste dehydrogenase as a result of which glucose and its intermediates enter into Polyol and Hexosamine pathways (Ceriello, 2011). The increased substrate flux through Polyol pathway results in decreased levels of NADPH and reduced glutathione (GSH).

There is compelling evidence that the increased generation of ROS causes oxidative stress leading to the onset of diabetes (Rosen *et al.*, 2001; Johansen *et al.*, 2005). Thus excessive ROS generation and other metabolic abnormalities (oxidation of fatty acids) are considered to play an important role in the development of Diabetes mellitus and its complications (Matough *et al.*, 2012). The fact that the levels of MDA in both type T1 and T2DM cases with good glycemic control in the present study still remains significantly higher than those in healthy subjects ($p<0.01$) indicates that apart from hyperglycemia there are other factors like oxidation of free fatty acid that may be contributing to excessive ROS generation.

Catalase is an important antioxidant enzyme which protects the cells from toxic effects of Hydrogen peroxide, generated during cell metabolism. It is present in all most all cells in human body but has been reported to be present in low quantities in pancreatic beta cells which explain high sensitivity of these cells to ROS. In view of afore mentioned reason catalase was selected in the present study in the serum of diabetic patients (Tiedge *et al.*, 1997). In this study there was significant decrease in the activity of serum catalase both in T1 and T2DM cases ($p<0.05$). This observation is supported by Goth (2008) who reported that catalase activity in T2DM cases was reduced compared with non-diabetics. Even in gestational diabetes significantly decrease in activity of this enzyme has been demonstrated (Goth *et al.*, 2006).

In a study, in T1 diabetic patients when blood glucose was strictly controlled, it was not accompanied by any remarkable changes in the prooxidant-antioxidant balance. Increased oxidative stress which also causes levels of circulating antioxidants to fall due to increased consumption is offered as an explanation for decreased catalase activity observed in the present study in diabetic patients (MacRury *et al.*, 1993). Glycation of the antioxidant enzymes which reduce their capacity to detoxify oxygen radicals may also be an important cause for the observed reduced catalase activity in our patients (Fatima *et al.*, 2012).

In view of the significant role of oxidative stress in pathogenesis of T1 and T2DM the use of antioxidants like Vitamin C, E and minerals has been recommended as supplement therapy. Diet rich in antioxidants can also be of help in slowing down the process of oxidative stress. However, in the absence of antioxidant therapy the use of statins, ACE inhibitors and angiotension receptor blockers has been suggested as preventive antioxidants (Ceriello, 2011).

The advancements made in unraveling molecular mechanisms of free radical generation in diabetes may lead to evaluation of antioxidant molecules that may mimic the activity of superoxide dismutase and catalase (Ceriello and Testa, 2009) and may effectively inhibit the molecular events leading to diabetic complications.

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