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Potential Role of Oxidative Stress and Antioxidant Deficiency in Pathogenesis of Diabetic Nephropathy

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Abstract

Introduction: Oxidative stress has been suggested to play a main role in the pathogenesis of type 2 diabetes mellitus and its micro vascular and macro vascular complications like nephropathy, retinopathy and neuropathy.

Aim: The aim of the study was to evaluate the magnitude of oxidative by measuring the lipid peroxidation and to assess the antioxidants status in patients with diabetes with and without nephropathy.

Materials and Methods: In the present study, 30 patients with Non insulin diabetes mellitus (NIDDM) without complication, 30 patients with type 2 diabetes mellitus with nephropathy and 30 clinically healthy individuals were evaluated. Lipid peroxidation in terms of serum malondialdehyde (MDA), by thiobarbituric acid method, erythrocyte superoxide dismutase (SOD), reduced glutathione (GSH) and serum vitamin C were estimated using spectrophotometer. Fasting blood glucose, Serum urea and creatinine were also measured.

Results: MDA content was significantly elevated in NIDDM patients without nephropathy when compared with the controls and even higher in NIDDM patients with nephropathy. SOD, GSH and Vitamin C levels were lower in NIDDM patients without nephropathy than in the controls and lowest in NIDDM patients with nephropathy.

Conclusions: Our findings indicate that changes in oxidant and antioxidant equilibrium will have biological and possibly pathological role in the development of secondary complications like nephropathy. Hence, we suggest that supplementation with dietary antioxidants especially antioxidant vitamins accompanied by change in lifestyle might help to reduce damage brought about by free radical toxicity in diabetes mellitus and its complications.

Key words: Ascorbic acid, Malondialdehyde, Reduced glutathione, Superoxide dismutase.

INTRODUCTION

Diabetic nephropathy is one of the major long-term complications of diabetes mellitus and has emerged as a leading cause of end-stage renal disease. According to the World Health Organization, diabetes affects more than 170 million people worldwide, and this number will rise to 370 million by 2030. [1] Data from the Diabetes Control and Complications Trial established hyperglycemia as the main determinant of initiation and progression of diabetic micro vascular complications like retinopathy, neuropathy, nephropathy etc.[2] among these complications, diabetic nephropathy affects 40% of type 1 and10% of type 2 diabetic patients. Diabetic nephropathy is a leading cause of end-stage renal failure worldwide. Its morphologic characteristics include glomerular hypertrophy, basement membrane thickening, mesangial expansion, tubular atrophy, interstitial fibrosis and arteriolar thickening. All of these are part and parcel of micro vascular complications of diabetes. A large body of evidence indicates that oxidative stress is the common denominator link for the major pathways involved in the development and progression of diabetic micro vascular as well as macro vascular complications of diabetes. Several lines of evidence suggest the central role of oxidative stress in the development of diabetic nephropathy and the beneficial effects of antioxidants in renal injury owing to diabetes.[3] Oxidative stress is defined as the excess formation and/or insufficient removal of highly reactive molecules (free radicals) such as reactive oxygen species and reactive nitrogen species.[4] It usually occurs when the available supply of the body's antioxidants is insufficient to handle and neutralize free radicals of different types. Oxidative stress free radicals are any substances that are capable of independent existence and that contain one or more unpaired electrons solely occupying an atomic orbit. [5] Because this molecular structure is energetically unstable, free radicals are highly reactive and extremely short-lived. Stability is achieved by the removal of electrons from that is the oxidation of surrounding substances to produce an electron pair. But the attacked substance then becomes a free radical, leading to propagation of a free radical chain reaction depending on

the reactivity of the target radical. The global term "reactive oxygen species" (ROS) includes both oxygen radicals such as superoxide (O^{2-}), alkoxyl (RO.), peroxyl (ROO) and hydroxyl radical (OH.) and non-radical derivatives of oxygen such as hydrogen peroxide (H_2O_2). Glucose directly increased H_2O_2 generation in mesangial cells leading to lipid peroxidation of glomeruli and mesangial cells in a dose dependent manner, which is highly supportive of the presence of increased oxidative stress in diabetic glomeruli.[5,6,7] Lack of effects of either L-glucose or mannitol on H_2O_2 generation and lipid peroxidation of mesangial cells suggest that high glucose-induced lipid peroxidation in this tissue is related not to an effect of high media osmolarity per se but to metabolism of glucose.[8,9] So it is suggested that, there is a high correlation between oxidative stress in diabetes and the development of complications including diabetic nephropathy. In diabetic patients, oxidative stress is evident within a few years of diagnosis before the onset of complications. As the disease progresses, antioxidant potential decreases, and the plasma lipid peroxidation products increase depending upon the level of glycaemic control. So the study was undertaken to assess the lipid peroxidation and key antioxidant enzymes in the erythrocytes of patients with diabetes with and without nephropathy.

MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry, JJM Medical College Davengere. Our study group included 30 patients of NIDDM without any complications with a mean age of 50.1±9.5 years, 30 patients of NIDDM with nephropathy with the mean age of 52.2 ± 6.5, 30 age and sex matched healthy controls with a mean age of 51.2± 12.4years. The diabetic patients were normotensive, without secondary causes of hyperglycemia and were under treatment with oral hypoglycemic agents. Detailed present and past history of the patients was collected on pre-tested performa which included name, age, sex, dietary habit, family history, smoking and drinking habit, socio-economic status, community and occupation along with their consent for the study. Selected subjects were asked to fast overnight and with all aseptic precautions blood sample was collected for estimation of fasting blood glucose, serum urea and creatinine, serum malondialdehyde (MDA), serum ascorbic acid (Vit C), erythrocyte SOD and reduced glutathione. Fasting blood glucose was estimated by O-Toluidine method.[10] Serum urea and creatinine were estimated.[10] Serum MDA was estimated by Thiobarbituric acid (TBA) method, in which one molecule of MDA reacts with two molecules of TBA and yields a pink crystalline pigment which is measured at 535 nm. [11] Serum ascorbic acid was estimated by 2,4 – dinitrophenyl hydrazine (DNPH) method in which ascorbic acid is oxidized by copper to form dehydroascorbic acid, which when treated with DNPH and sulfuric acid forms orange colour which is measured at 520 nm. [12] Reduced glutathione was estimated by 5,5 dithiobis – 2 – nitrobenzoic acid (DTNB) method. DTNB is readily reduced by sulphydryl compounds, forming a highly coloured yellow anion. Optical density is measured at 412 nm.[13] Superoxide dismutase in hemolysate was estimated using Nitroblue Tetrazolium (NBT). Illumination of riboflavin in the presence of oxygen and electron donors like methionine or EDTA generates superoxide anion. The reduction of nitroblue tetrazolium by O_2^- was followed at 560 nm using a spectrophotometer. [14]

STATISTICAL ANALYSIS

The statistical results are expressed as Mean ± SD. The comparison of the results of patients and healthy controls was done by performing unpaired t-test and the statistical significance was determined from the p value. Lipid peroxidation and the antioxidant vitamin status were correlated with glycaemic control in patients with diabetic nephropathy by calculating the Pearson's coefficient of

Table 1. Comparison of FBS, MDA, Vit. C, GSH and SOD between controls, NIDDM without complications and NIDDM with nephropathy

Groups	*FBS (mg/dl)*	*Serum Urea (mg/dl)*	*Serum creatinine (mg/dl)*	*MDA (nmol/ml)*	*Vit. C (mg/dl)*	*GSH (mg/dl)*	*SOD (U/ml)*
Controls (I) N=30	98.1± 7.40	18.2± 4.41	0.81 ± 0.25	3.91± 0. 54	1.72 ± 0.21	58.4± 3.53	5.51 ± 1.46
NIDDM without complications(II) N=30	209.5± 23.50	24.1± 4.83	1.02 ±0.2 42	4.78 ± 0.76	0.94 ± 0.32	46.1± 3.89	3.89 ± 0.49
NIDDM with Nephropathy(III) N=30	263.7± 10.82	64.56±2.48	2.50 ±3.47	6.77± 0.44	0.85 ± 0.23	38.44±2.69	2.97± 0.23
*I versus II	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p< 0.001
*I versus III	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p< 0.001	p< 0.001	p < 0.001
*II versus III	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p< 0.001	p < 0.001	p< 0.001

All values expressed as mean ± SD
*Unpaired‘t’ test p > 0.05, Not Significant (NS), p < 0.05, p < 0.01Significant (S),

correlation (r value) and the statistical significance was determined from the p value.

RESULTS

The results obtained from controls, diabetic without complication and diabetic with nephropathy groups are shown statistically in Table 1. Vit C, GSH, SOD levels of diabetics without nephropathy are significantly lower (P<0.001), while FBS, serum MDA levels were significantly higher, when compared with those of control group (P<0.001). Table 1 FBS, Serum urea, creatinine, serum MDA levels of the group with nephropathy were significantly higher (P<0.001, whereas Vit C, GSH, SOD levels were significantly lower relative to those of the group without nephropathy (P<0.001). (Table 1) FBS, Serum urea, creatinine, serum MDA levels of the group with nephropathy were significantly higher (P<0.001) Vit C, GSH, SOD levels were significantly lower, when compared to control group (P<0.001). Mean Percentage change of oxidant and antioxidant parameters in patients with diabetic nephropathy compared to controls are shown in Fig.1. A statistically significant positive correlation was found between FBS and MDA, r = 0.8787 shown in Fig.2. A statistically significant negative correlation was found between FBS and Vit C, r = -0.7302 shown in Fig 3, FBS and GSH, r = -0.8028 in Fig. 4 FBS and SOD, r = -0.7407 in Fig 5 in diabetic nephropathy.

Fig. 1. Percentage change of mean oxidant and antioxidant parameters in patients with diabetic nephropathy compared to controls.

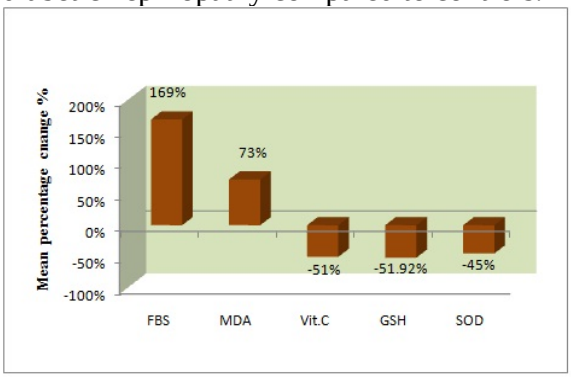


Fig.2. Correlation between FBS (mg/dl) and MDA (nmol/ml) in diabetic nephropathy

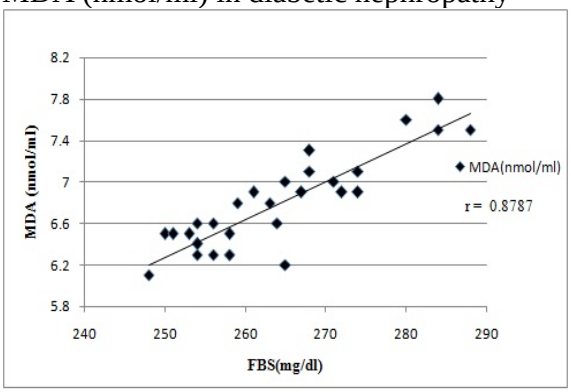


Fig.3.Correlation between FBS (mg/dl) and Vit.C (mg/dl) in diabetic nephropathy

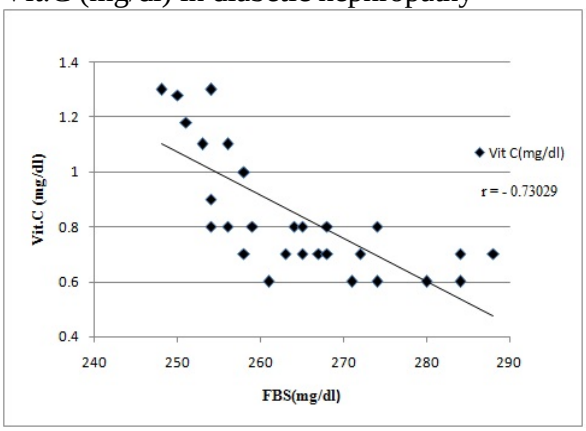


Fig.4. Correlation between FBS (mg/dl) and GSH (mg/dl) in diabetic nephropathy

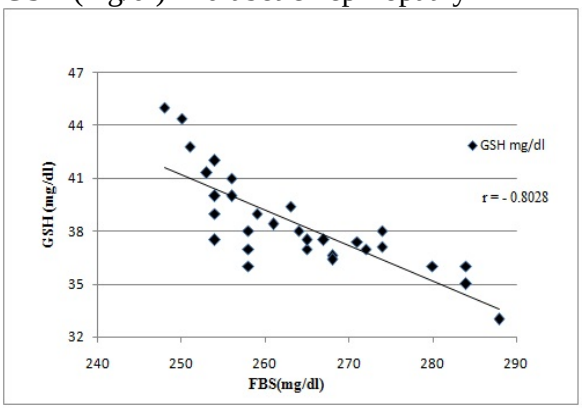
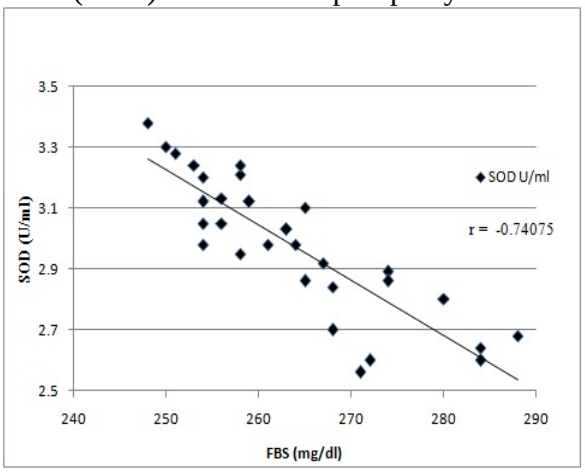


Fig.5. Correlation between FBS (mg/dl) and SOD (U/ml) in diabetic nephropathy



DISCUSSION

Diabetes mellitus is a complex and multifactorial disease indulging severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis, lipid and protein metabolism. The metabolic deregulations associated with diabetes causes secondary pathophysiologic changes in multiple organ systems that impose a heavy burden of morbidity and mortality from macro vascular and micro vascular complications.[15]

Accumulating research suggests that oxidative stress is a significant contributor to the pathogenesis of diabetic nephropathy. The normal kidney generates a substantial amount of oxidative stress because of its high metabolic activity that is balanced by an extensive antioxidant system. However, in pathologic states such as hyperglycaemia, oxidant balance shifts toward a pro-oxidant state that accelerates tissue and vascular injury. This oxidative damage progresses concomitant with worsening glucose metabolism, vascular dysfunction and kidney disease. Accordingly, strategies to reduce oxidative stress in diabetes mellitus may exert favorable effects on the progression of diabetic nephropathy. [16]

A significant increase in MDA in patients with NIDDM was found compared with the control group which suggested permanent structural membrane alterations in diabetes and also increased production of reactive oxygen species in the circulation. This has been demonstrated by other authors.[17]
In our study we have also observed further intensification of lipid peroxidation takes place in NIDDM patients with obvious nephropathy compared with the group without nephropathy. This fact may indicate increased production of free radicals or diminished efficiency of antioxidant defense mechanisms in diabetic nephropathy.[18]

Increased ant oxidative glycosylation of haemoglobin may lead to imbalanced generation of free radicals like superoxide, thereby causing depletion of SOD which quenches it. Diminished activity of SOD points out to an exhausted antioxidant reserve

which further exacerbates the oxidative stress. Excessive peroxidation is associated with reduced SOD activity in diabetes. Loss of SOD activity in the erythrocytes appears to be a function of the duration of diabetes. SOD, inhibited by glycosylation, is lowered in poorly controlled diabetes mellitus. Due to the absence of protein synthesizing machinery in the erythrocytes, the inactivation SOD by glycosylation may be dominant factor in the loss of SOD activity observed. SOD deficiency is seen within 2 years of the detection of NIDDM and further decrease with development of complications.[19] Our results confirm this conclusion, since significantly decreased activity of SOD was found in NIDDM patients compared to the controls. A further decrease in the activity of SOD was observed in NIDDM patients with nephropathy.

In diabetic subjects, the increased sorbitol synthesized caused NADPH depletion, which when deficient, limits the reduction of GSSG to GSH. Therefore, a major decrease in GSH may profoundly impair free radicals scavenging activity, resulting in exacerbated cell damage after exposure to free radical generated by glucose autoxidation. The levels of glutathione are regulated by glutathione peroxidase and glutathione reductase. The decreased activity of glutathione reductase in diabetics together with the decreased transport rate of GSSG indicates that regeneration and transport systems, which decrease intracellular GSSG, are impaired in diabetics, when erythrocytes are exposed to oxidative stress.[20] In our study we have found out decrease GSH in patients with diabetes without nephropathy and the decrease is more in patients with diabetic nephropathy.

Water-soluble vitamin C and fat-soluble vitamin E together make up an antioxidant system for mammalian cells. Vitamin C, or ascorbic acid, is considered the most important antioxidant in plasma and forms the first line of defense against plasma lipid peroxidation.[21]

Ascorbic acid participates in many cellular oxidation–reduction reactions including hydroxylation of polypeptide lysine and proline residues and dopamine that are required for collagen production and metabolism and storage of catecholamines in neurons.Increase in the oxidative stress level and metabolic perturbations can be expected in any tissue or cell type that relies exclusively or mainly on GLUT for co-transport of glucose and DHA including neurons, epithelial cells, and vascular tissues. [22] On the other hand, since DHA represents a significant proportion of total serum ascorbate, by increasing total plasma ascorbate concentrations during hyperglycemia, it should be possible to correct the increase in the oxidative stress level and metabolic perturbations, thereby sparing diabetic patients many of their complications.[22] As vitamin C is one of the major contributors to serum total antioxidant activity our results indicate that diabetic patients have significant defects in antioxidant protection, which may increase the vulnerability to oxidative damage and the development of diabetic complications such as diabetic nephropathy.

CONCLUSION

In conclusion, free radical formation along with antioxidant deficiency in diabetes mellitus increases over time and may play an important role in the development of diabetic nephropathy, which is an important complication of the disease. The present study revealed the importance of determining the antioxidant status in diabetes, in addition to the markers of oxidative stress, to enable the formulation of specific therapies for an early intervention and better management of the disease and its complications.

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