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RESEARCH ARTICLE

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PROXIDANT AND ANTIOXIDANT STATUS IN TYPE 2 DIABETES WITH RELATION TO ITS DURATION

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ABSTRACT

Diabetic patients are exposed to increased oxidative stress due to several mechanisms, including glucose auto-oxidation and non enzymatic protein glycation. The aim of the present study was to evaluate the levels of selected oxidation and antioxidant status in type 2 diabetes and to correlate the lipid peroxidation and antioxidant with duration of diabetes and glycemic control. Degree of lipid peroxidation in terms of serum malondialdehyde (MDA), along with enzymatic antioxidants (ie, superoxide dismutase (SOD) and free radical scavengers ie, reduced glutathione (GSH) and vitamin C were estimated in 60 controls and 89 type 2 diabetic patients. Analysis was done using Unpaired't' test, for comparison between two groups and One way ANOVA (F-test) for multiple group comparison. FBS, serum MDA levels of diabetic patients were significantly higher ($p<0.001$), whereas SOD, GSH, Vit C, levels were significantly lower relative to their controls ($p<0.001$). There was a highly significant $p<0.001$ increase in the mean levels of FBS and MDA in NIDDM patients whereas SOD, GSH, Vit C shows a statistically significant decrease with the both glycemic control and the duration of diabetes. Certain indices of oxidant stress are influenced by the duration of diabetes and the efficacy of glycemic control. These observations suggest that supportive therapy aimed at oxidative stress may help to prevent clinical complications in type 2 diabetes mellitus.

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KEYWORDS

Ascorbic acid, Malondialdehyde, Reduced glutathione, Superoxide dismutase

INTRODUCTION

Diabetes is a devastating disease throughout the world. It has been estimated that the number of people affected with diabetes in the world will increase to 300 million by 2025. Diabetes is characterized by chronic hyperglycemia that produces deregulation of cellular metabolism. Diabetes overloads glucose metabolic pathways, resulting in excess free radical production and oxidative stress.[1] Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both.[2] Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to non enzymatic protein glycosylation, auto-oxidation of glucose,[3] impaired glutathione metabolism,[4] alteration in antioxidant enzymes[5], lipid peroxides formation[6] and decreased ascorbic acid levels. [7] In addition to GSH, there are other defense mechanisms against free radicals like the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals.[8] 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 glycemic control. The purposes of this study were as follows, to analyze the nature and extent of oxidative damage in type 2 diabetes mellitus, compared to control subjects and to evaluate the influence of diabetes duration and glycemic control on the free-radical indices and free radical scavengers in diabetic patients. Degree of lipid peroxidation in terms of serum malondialdehyde (MDA), along with enzymatic antioxidants (ie, superoxide dismutase (SOD) and free radical scavengers ie, reduced glutathione (GSH) and vitamin C were estimated.

MATERIALS AND METHODS

The study population consisted of 149 subjects (age-matched subjects) divided into two groups viz., diabetic patients (type 2 diabetic subjects; n=89) and healthy controls (n=60). The study was carried out at Bapuji Hospital, Davangere. General health characteristics such as age, sex, smoking status, menopausal status, alcohol consumption and dietary habits, particularly as related to preference were investigated by a self-administered questionnaire. Overnight fasting blood sample was collected for estimation of fasting blood glucose, serum MDA, serum ascorbic acid, erythrocyte SOD and reduced glutathione. Fasting blood glucose was estimated by O-Toluidine method. [9] 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.[10] 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. [11] Blood reduced glutathione was estimated by 5,5 dithiobis – 2 –nitrobenzoic acid (DTNB) method. DTNB is

readily reduced by sulphydryl compounds, forming a highly colored yellow anion. Optical density is measured at 412 nm.[12] Superoxide dismutase in hemolysate was estimated using Nitroblue Tetrazolium (NBT). Illumination ofriboflavin 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 560nm using a spectrophotometer. [13]

STATISTICAL ANALYSIS:

All the values are expressed as their Mean ± S.D. Data were subjected for analysis using Unpaired 't' test, for comparison between two groups and One way ANOVA(F-test) for multiple group comparison.

RESULTS

Degree of lipid peroxidation in terms of serum malondialdehyde (MDA), along with enzymatic antioxidants ie, superoxide dismutase (SOD) and free radical scavengers ie, reduced glutathione (GSH) and vitamin C between controls and type 2 diabetes was shown in Table 1. FBS, serum MDA levels of diabetic patients were significantly higher ($p<0.001$), whereas SOD, GSH, Vit C, levels were significantly lower relative to their controls ($p<0.001$). (Table 1). Table 2 compares the mean levels of FBS, MDA, SOD, GSH and Vit. C in type 2 diabetes patients depending on the duration of diabetes mellitus. It is evident from the table there is a highly significant ($p<0.001$) increase in the mean levels of FBS and MDA in type 2 diabetes patients as the duration of diabetes goes on increasing whereas SOD, GSH, Vit C shows a statistically significant decrease as the duration of diabetes goes on increasing. Table 3 compares the mean levels of FBS, MDA, SOD, GSH and Vit C in in type 2 diabetes patients depending on glycemic control. It is evident from the table there was a highly significant $p<0.001$ increase in the mean levels of FBS and MDA in type 2 diabetes patients whereas SOD, GSH, Vit C shows a statistically significant decrease as the glycemic control is poor.

Table 1

Comparison of FBS, MDA, SOD, GSH and Vit .C between controls and Type 2 diabetes

Groups	No	FBS (mg/dl)	MDA (nmol/ml)	SOD (U/ml)	GSH (mg/dl)	Vit. C (mg/dl)
Controls	60	94.7 ± 6.4	3.62 ±0.24	5.31 ±1.04	55.3 ± 3.15	1.67 ± 0.20
Type 2 diabetes	89	230.0±33.0	5.14± 0.68	3.72 ±0.46	46.92±2.49	0.79 ± 0.19
*t-value		23.45	11.10	23.2	12.8	10.74
p-value		0.001	0.001	0.001	0.001	0.001
Significance		HS	HS	HS	HS	HS

Values are expressed as Mean ± S.D
**Unpaired 't' test*

Table 2
Comparison of FBS, MDA, SOD, GSH and Vit C in Type 2 diabetes patients according to the duration of diabetes

Duration of Diabetes	No. of subjects	FBS (mg/dl)	MDA (nmol/ml)	SOD (U/ml)	GSH (mg/dl)	Vit. C (mg/dl)
< 5 years	17	188.0± 15.8	4.19 ± 0.25	4.19 ± 0.42	50.68± 1.5	1.09 ± 0.19
5-10 years	15	205.6± 15.5	4.78± 0.34	3.96 ± 0.59	46.73 ± 2.12	0.86 ± 0.11
11- 15 years	26	227.2± 8.7	5.20 ± 0.15	3.59 ± 0.24	46.43 ± 1.26	0.673 ± 0.06
>15 years	31	253.1± 15.5	5.64±0.20	3.23± 0.24	45.41± 1.71	0.61 ± 0.07
*F – value		70.5	54.7	17.8	46.1	56.7
p – value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values are expressed as Mean ±S.D
**ANOVA (F- test)*
p < 0.001 –Highly significant

Table 3
Analysis of FBS, MDA, SOD, GSH and Vit. C between controls and Type 2 diabetes patients depending on the glycemic control

Groups	No. of cases	MDA (nmol/ml)	SOD (U/ml)	GSH (mg/dl)	Vit. C (mg/dl)
A	18	4.29 ± 0.33	4.17 ± 0.41	50.7 ± 1.7	1.04 ± 0.27
B	45	5.18 ± 0.48	3.71 ± 0.44	46.8 ± 2.2	0.78 ± 0.12
C	26	5.66 ± 0.59	3.43 ± 0.23	44.5 ± 1.6	0.65 ± 0.05
F – value		41.7	19.4	54.0	43.3
p – value		< 0.001	< 0.001	< 0.001	< 0.001

Groups A – FBS < 200 mg/dl B – FBS 200 – 250 mg/dl C – FBS > 250 mg/dl
Values are expressed as Mean ±S.D
**ANOVA (F- test)*
p < 0.001 –Highly significant

DISCUSSION

Diabetes mellitus (DM) is associated with endothelial dysfunction and oxidative stress. Chronic exposure to elevated glucose and fatty acid concentrations can cause damage in different types of cells by a variety of mechanisms collectively known as glucolipotoxicity, and oxidative stress may be a common link. [1] The oxidative stress in DM is greatly increased due to prolonged exposure to glycemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of oxidative stress.[3] Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of DM.[2]

Our study showed an increase in MDA levels in DM patients, which is in keeping with the results of previous studies. Increased non-enzymatic and autooxidative glycosylation is one possible mechanism for free radical-induced lipid peroxidation in DM. Some authors have found a positive correlation between the MDA level and indices of glycaemic control and our results are in support of this. [14] Pasaoglu et al in their study on 20 patients with newly diagnosed DM and 20 patients with a mean

DM duration of seven years, patients in the latter group had higher serum and erythrocyte MDA concentrations and lower levels of glutathione.[15]

Our study showed a decrease reduced glutathione in diabetes and negative correlation between duration and glycemic control. Sushil K. Jain and Robert McVie showed that erythrocytes of diabetic patients have a significantly lower glutathione level compared with those of age-matched normal subjects. They also found a significant negative correlation between the degree of hyperglycemia and the level of reduced glutathione (GSH) in erythrocytes of diabetic patients. Using erythrocytes as a model, their study suggests that a lower level of GSH may have a role in the cellular damage and impaired insulin secretion in uncontrolled diabetic patients.[16]

Study on serum superoxide dismutase in type-2 diabetes mellitus showed that superoxide dismutase levels are decreased in type 2 diabetes mellitus and with duration of diabetes mellitus and our study goes along with them. A negative correlation between the erythrocyte activity of SOD and glycemic control can be observed in the present study. This is suggestive of the fact that increased autoxidation glycosylation of hemoglobin may have lead enhanced generation of free radicals like the super oxide anion, thereby causing the depletion of SOD which quenches it. Tare RS and co-workers illustrated the prevalence of oxidative stress in diabetes by a highly significant increase in concentration of MDA and diminished activity of SOD in comparison to the control subjects and observed a negative correlation between the erythrocyte activity of SOD and glycemic control.[17] A likely explanation for a lower Vitamin C status in diabetics is that ascorbic acid is actively transported into the cells in its partially oxidized form as dehydroascorbic acid, which is promptly converted to ascorbic acid within the cell. In our study vitamin C showed an inverse correlation with the both glycaemic control and the duration of diabetes. Similar findings was reported by Sundaram et al in their study on antioxidant in diabetes, [18] The carrier of ascorbic acid transport serves also to transport glucose and is inhibited in transporting ascorbic acid by the hyperglycemia of diabetics. Lysy et al showed that in diabetics, plasma ascorbic-acid levels were negatively correlated with glycosylated hemoglobin, a measure of glycemic control.[19]

CONCLUSION

The results of the present study and previous reports provide ample evidence that oxidant stress is present in patients with type 2 diabetes mellitus. Moreover, certain indices of oxidant stress and antioxidants are influenced by the duration of diabetes and the efficacy of glycemic control. These observations suggest that supportive therapy aimed at oxidative stress may help to prevent clinical complications in type 2 diabetes mellitus.

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