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ORIGINAL ARTICLE

Impaired Antioxidant Defence Mechanism In Diabetic Retinopathy

T.VIVIAN SAMUEL, JAYAPRAKASH MURTHY D S K, DATTATREYA , P SURESH BABU, S SMILEE JOHNCY

ABSTRACT

Background

Oxidative stress resulting from enhanced free-radical formation and a defect in antioxidant defences, has been implicated in the pathogenesis of diabetes and its complications include neuropathy, retinopathy, nephropathy, etc.

Aims

The aim of the present study was to evaluate the levels of selected oxidation and the antioxidant status with relevance to diabetic retinopathy and to correlate the antioxidant status with glycaemic control.

Materials and Methods

Thirty patients of type-2 diabetes mellitus (DM) without retinopathy, 30 patients of diabetic retinopathy and 30 normal subjects were included in this study. The degree of lipid peroxidation in terms of serum malondialdehyde (MDA) by the thiobarbituric acid method along with antioxidant defences, erythrocyte superoxide dismutase (SOD), reduced glutathione (GSH) and serum vitamin C was estimated in healthy controls and non-insulin dependent diabetes mellitus (NIDDM) subjects with and without retinopathy. Fasting blood glucose was also estimated and correlated with the antioxidant status. The statistical analysis was done by using the Students unpaired 't' test and the correlation was done by using Pearson's correlation coefficient.

Results

The levels of serum MDA were found to be increased significantly in the diabetic retinopathy cases as compared to those in the cases with diabetes without retinopathy and in the healthy controls. The levels of superoxide dismutase (SOD), reduced glutathione (GSH) and vitamin C were significantly reduced in all diabetic patients, i.e., those with and without retinopathy. However, the lowest levels were found in the diabetic patients with retinopathy. A negative correlation was found between FBS and antioxidant enzymes in the diabetic retinopathy cases.

Conclusion

The results suggest that antioxidant deficiency and excessive peroxidation damage appear in NIDDM, and the severity is more with the development of complications. Therapeutic measures to increase the antioxidants and to control lipid peroxidation are warranted for the effective control of its complications.

Keywords: Ascorbic acid, Malondialdehyde, Reduced glutathione, Superoxide dismutase.

Keymessage: Raised oxidative stress, decreased antioxidant status and antioxidant deficiency appears to be associated with a risk for diabetic retinopathy.

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INTRODUCTION

Retinopathy is one of the most severe ocular complications of diabetes and is a leading cause of acquired blindness in young adults. A prominent and early feature of the retinopathy of diabetes mellitus is a diffuse increase in vascular permeability. As the disease develops, the development of frank macular oedema may result in vision loss. The cellular components of the retina are highly coordinated but are very susceptible to the hyperglycaemic environment. [1] Oxidative stress has been implicated in the pathogenesis of diabetic retinopathy. It has been hypothesized that hyperglycaemia may damage the vascular endothelium and the retina by inducing the synthesis of oxidant reactive species and thereby causing oxidative stress. [2] Oxidative stress is defined as an increase in the steady-state levels of reactive oxygen species. Free radicals are very reactive chemical species and can cause oxidative injury to living beings by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and the antioxidant defence systems which are used by organisms to deactivate and protect themselves against free radical toxicity. [2] Impairment in the oxidant/antioxidant equilibrium creates an oxidative stress which is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases. [3] There are several endogenous enzyme systems that protect the cell and tissue from oxidative stress, for example superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px). Although there is controversy about the antioxidant status in diabetes, several studies report decreased levels of SOD and GSH-Px in both clinical and experimental diabetes, indicating an impaired defence system for free radical scavenging. The sources of reactive oxygen species in diabetes may include the autoxidation of glucose. [4] As oxidative stress plays an important role in the chronic complications of diabetes, it is postulated to be associated with increased lipid peroxidation. Hyperglycaemia and dyslipidaemia in diabetes mellitus induce increased lipid peroxidation and peroxyl radical formation which are important mechanisms in the genesis of microangiopathy. In this study, malondialdehyde (MDA) levels as an index of erythrocyte susceptibility to oxidative stress and antioxidant status in type – 2 DM patients with and without retinopathy, was studied and it was compared with a control non-diabetic group.

MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry, JJM Medical College, Davengere. Our study group included 30 patients of DM type-2, without any complications, with a mean age of 50.6±10.5 years, 30 patients of DM type-2 with retinopathy, with a mean age of 49.5±11.7 years and 30 age and sex matched healthy controls with a mean age of 50.8 ± 14.8 years. The diabetic patients were normotensive, without the secondary causes of hyperglycaemia and were under treatment with oral hypoglycaemic agents. The detailed present and past history of the patients was collected on a pre-tested performa which included name, age, sex, dietary habits, family history, smoking and drinking habits, socio-economic status, community and occupation, along with their consent for the study. An experienced ophthalmologist performed the direct

opthalmoscopic examination on the patients. Retinopathy was defined as mild to moderate non - proliferative, severe non - proliferative and clinically significant macular oedema. The selected subjects were asked to fast overnight and with all aseptic precautions, blood samples were collected from them for the estimation of fasting blood glucose, serum malondialdehyde (MDA), serum ascorbic acid (Vit C), erythrocyte SOD and reduced glutathione. Fasting blood glucose was estimated by the O-Toluidine method. [5] Serum MDA was estimated by the 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. [6] Serum ascorbic acid was estimated by the 2, 4 – dinitrophenyl hydrazine (DNPH) method in which ascorbic acid is oxidized by copper to form dehydroascorbic acid, which when treated with DNPH and sulphuric acid, forms an orange colour which is measured at 520 nm. [7] Reduced glutathione was estimated by the 5, 5 dithiobis – 2 – nitrobenzoic acid (DTNB) method. DTNB is readily reduced by sulphydryl compounds, forming a highly coloured yellow anion. Optical density was measured at 412 nm. [8] Superoxide dismutase was estimated in haemolysates by using Nitroblue Tetrazolium (NBT).[9] The illumination of riboflavin in the presence of oxygen and electron donors like methionine or EDTA generates superoxide anions. The reduction of nitroblue tetrazolium by O_2^- was read at 560 nm by using a spectrophotometer. [10]

Statistical analysis

All the values were expressed as their Mean ± S.D. The data were analysed by using the students unpaired‘t’ test for comparison between the two groups and the correlation between glycaemic control and the antioxidants was done by using Pearson's correlation coefficient.

RESULTS

The results obtained from the control, diabetics without complications and diabetics with the retinopathy groups, are shown statistically in [Table/Fig 1]. The levels of Vit C, GSH and SOD of diabetics without retinopathy were significantly lower (P<0.001), while the FBS and serum MDA levels were significantly higher, when compared with those of the control group (P<0.001) [Table/Fig 1].

The levels of FBS and serum MDA of the group with retinopathy were significantly higher (P<0.001), whereas the levels of Vit C, GSH and SOD were significantly lower with respect to those of the group without retinopathy (P<0.001) [Table/Fig 1]. The levels of Vit C, GSH and SOD were significantly lower in the cases of diabetes with retinopathy when compared to those of the control group (P<0.001) [Table/Fig 1].

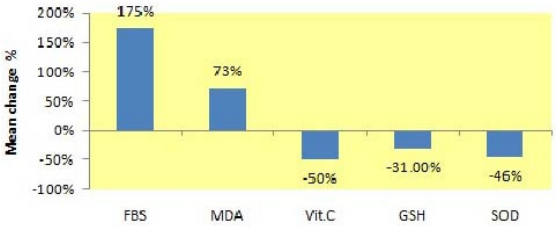
[Table/Fig 1]: Comparison of FBS, MDA, Vit C, GSH and SOD between controls, NIDDM without complications and NIDDM with retinopathy

Groups	No. of cases	FBS (mg/dl)	MDA (nmol/ml)	Vit. C (mg/dl)	GSH (mg/dl)	SOD (U/ml)
Controls (I)	30	94.7±6.7	3.89 ± 0. 37	1.61 ± 0.10	56.3 ± 3.42	5.48 ± 1.12
NIDDM without complications (II)	30	217.8 ± 18.4	4.63± 0.58	0.91 ± 0.20	49.6 ± 3.01	3.97± 0.49
NIDDM with Retinopathy III	30	260.7± 7.06	6.72± 0.20	0.80 ± 0.05	39.08±2.73	3.01 ± 0.22
*I versus II		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
*II versus III		p < 0.001	p < 0.001	p< 0.001	p < 0.001	p< 0.001

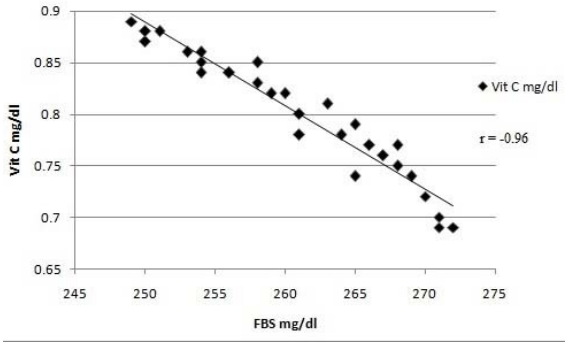
All values expressed as mean ± SD
***Unpaired ‘t’ test**
P > 0.05, Not Significant (NS), P < 0.05, P < 0.01Significant (S),
P < 0.001, Highly Significant (HS)

The mean percentage changes of the oxidant and antioxidant parameters in patients with diabetic retinopathy as compared to the controls are shown in [Table/Fig 2]. A statistically significant negative correlation was found between FBS and Vit C, r = -0.9629 shown in [Table/Fig 3], between FBS and GSH, r = -0.8422 in [Table/Fig 4], and between FBS and SOD, r = -0.9081 in [Table/Fig 5] in cases of diabetic retinopathy.

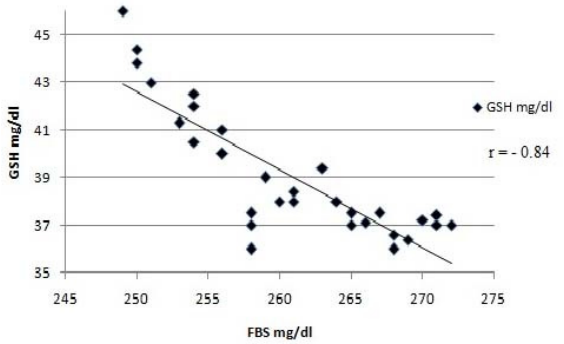
[Table/Fig 2]: Percentage change of mean oxidant and antioxidant parameters in patients with diabetic retinopathy compared to controls



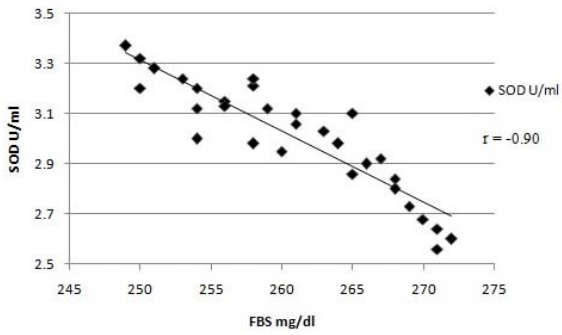
[Table/Fig 3]: Correlation between FBS (mg/dl) and Vit C (mg/dl) in diabetic retinopathy



[Table/Fig 4]: Correlation between FBS (mg/dl) and GSH (mg/dl) in diabetic retinopathy



[Table/Fig 5]: Correlation between FBS 9mg/dl) and SOD (U/ml) in diabetic retinopathy



DISCUSSION

Diabetic retinopathy is perhaps the most specific of all the diabetic complications. The prevalence of retinopathy is related to the duration of diabetes. Rema et al. studied the prevalence of retinopathy in 6782 NIDDM patients, in which the overall prevalence was found to be 34.2%, of which 30.8% was non- proliferative diabetic retinopathy and 3.4% was proliferative diabetic retinopathy. In the same study, the prevalence of non- proliferative diabetic retinopathy and proliferative diabetic retinopathy were 7.2% and 0.2% at the onset of diabetes, which increased to 73% and 11.9% after 20 years of duration of diabetes. [10]

There has been a great deal of interest in the role of oxidant stress in the causation of tissue damage in a number of diseases. It is also proved that hyperglycaemia may damage the vascular endothelium and the retina by inducing the synthesis of oxidant reactive species. Oxidant stress is usually countered by an abundant supply of antioxidants. If concomitant antioxidant deficiency occurs, oxidant stress may produce tissue damage. Normally, the body has an abundant supply of "antioxidants" which are naturally occurring substances that delay or inhibit oxidation and neutralize the oxygen free radicals. In the nature, therefore, when there is a balance of "oxidant stress" and the "antioxidant supply", there is perfect harmony and no tissue destruction occurs. However, if there is an imbalance, i.e., either an excess of free radicals and reactive oxygen species or a deficiency of antioxidant supply, then tissue damage can occur. [11]

In our study, we found increased lipid peroxidation in terms of MDA in diabetics as compared to the controls and this increase was more in diabetic retinopathy. Gallau and his colleagues demonstrated that lipid peroxides (MDA) were higher in diabetics with retinopathy as compared to those in diabetics without retinopathy. [12] Yıldız and his coworkers also identified higher plasma and erythrocyte MDA levels in the group with retinopathy, as compared with those in the group without retinopathy. There are a few biochemical mechanisms that explain the reason for this rise. The increase in the blood free fatty acid levels depending on degree of lipolysis, results in an increase in MDA production. Increased MDA levels of diabetic individuals may take origin from the peroxidative damage of the membrane lipids. [13] Jennings et al. (1991) have shown that increased free radical activity leads to an increased thrombotic tendency and a reduction in prostacyclin stimulating factors depending on the increasing thrombocyte reactivity in diabetics, especially with retinopathy, and they have also demonstrated that intracellular SOD activity is reduced in patients with retinopathy. [14] Since lipid peroxides play a major role in the formation of vascular tissue damage, it is suggested that an increase in MDA in diabetes can be effective in the pathogenesis of diabetic angiopathy. It has been indicated that an increase in free fatty acids along with a lack of insulin contribute to high plasma MDA levels. [15]

One of the intracellular protective mechanisms against free radicals that form peroxidation in the membrane lipids is the glutathione and the redox systems. Reduced GSH is a non-specific reduction agent and it plays an important role in the oxidation mechanisms. [16] In cell metabolism, it performs important functions in the prevention of the sulphydryl groups of various proteins and lipoproteins in the cell membrane. GSH participates in antioxidative defense systems as a free radical inactivator. GSH is maintained at a concentration of 0.2-10 mM in all mammalian cells. Depletion of GSH in the cell renders it susceptible to oxidative injury. [16] Under in vivo conditions, GSH acts as an antioxidant and its decrease is reported in diabetes. Rathore et al. (2000) have observed a significant decrease in the GSH content in diabetic erythrocytes. The decrease in GSH content represents increased utilization due to oxidative stress. The depletion of GSH levels may also lower the GST activity, as GSH is required as a substrate for GST activity. Depression in the GPx activity was also observed in erythrocytes during diabetes. GPx has been shown to be an important adaptive response to and condition of increased peroxidative stress. [17]

The weakness of the antioxidant defence system may be the biochemical background for the pathogenesis of the endothelial dysfunction which is associated with diabetes. Ascorbic acid functions as an important component of the cellular defence against oxygen toxicity and lipid peroxidation which is caused by the free radical mechanism. Reduced levels and the altered metabolic turnover of ascorbic acid have been reported in diabetic patients. [18] A decrease in the plasma concentration of ascorbic acid has been observed in diabetic patients. In our study, we found decreased levels of Vitamin C in diabetic patients as compared to the controls and this decrease was found to be more in diabetic retinopathy. The uptake of ascorbic acid into the cell is mediated by processes which are related to glucose transport and it has been shown that the high extracellular glucose concentration in diabetes may further impair the cellular uptake of ascorbic acid and accentuate the problems which are associated with its deficiency. Vitamin C is a hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to the reactive oxygen species. The observed significant decrease in the levels of plasma vitamin C could be caused by the increased utilization of vitamin C as an antioxidant defence against the reactive oxygen species or by a decrease in GSH, which is required for the recycling of vitamin C. Earlier research has shown that diabetics have low levels of vitamin C and that vitamin supplementation can help in preventing the development of glucose intolerance and diabetes [19].

Vitamin C is an important component of the cell defence systems working against oxidative stress. These levels are significantly decreased in diabetics and more so, in diabetic retinopathy. Our study also suggested similar findings. The uptake of ascorbic acid into the cell is mediated by processes which are related to glucose transport and it has been shown that the high extracellular glucose concentration in diabetics may further impair cellular uptake and accentuate the problems which are associated with its deficiency. [17] Also, therapeutic doses of vitamin C are associated with the reversal of early signs of retinopathy in diabetics, thus confirming its protective role in the damage of the blood vessels. Thus, the increase in lipid peroxides in blood, coupled with the weakness of the defense antioxidant system in diabetics without complications, probably serves as a background for the pathogenesis of endothelial dysfunction which is associated with diabetes. [19]

A decrease in the activities of the antioxidant enzyme systems in diabetes is linked to the progressive glycation of the enzymatic proteins. Numerous reports indicate variations in the levels of antioxidants in the diabetic patients. [2] In our study, it was found that the levels of SOD, an enzyme which is responsible for the scavenging of oxidant stress factors in the body, is significantly decreased in diabetics with and without retinopathy. The superoxide anion which is believed to be one of the initiators of free radical reactions plays an important role in the determination of the levels of the antioxidant enzyme, SOD. The products of membrane lipid peroxidation and other oxidants like H_2O_2 may react with SOD, resulting in oxidative modification, thereby causing loss of enzyme activity. Also, diabetic hyperglycaemia leads to the glycation and inactivation of SOD, thus attributing to its decrease. [20]

These results indicate that there occur severe lipid peroxidation, protein oxidation, and oxidative DNA damages in diabetes. The augmented oxidative stress in diabetes may be speculated to contribute to the pathogenesis of diabetes mellitus. Oxidative stress is an important risk factor in the development of diabetic retinopathy. The present study also showed that type 2 diabetic patients have retinopathy as a complication and also have a low antioxidant status as compared to those of healthy individuals.

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 retinopathy, 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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