Research Article

Appraisal of oxidative stress markers and antioxidant status in diabetic neuropathy

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Abstract

Introduction: Oxidative stress resulting from enhanced free-radical generation and/or a decrease in antioxidant defenses has been implicated in the pathogenesis of diabetic neuropathy. This study was conducted to evaluate oxidative stress and antioxidant balance in diabetic neuropathy and to correlate this with glycemic control. **Method:** Thirty patients with diabetic neuropathy and thirty age matched healthy controls were included in the study. Fasting blood glucose and glycosylated hemoglobin (HbA1C) were estimated to assess the severity of diabetes and the glycemic control respectively. Serum malondialdehyde (MDA) levels were assessed as a marker of lipid peroxidation and hence oxidative stress. Erythrocyte glutathione peroxidase levels were assessed for antioxidant status. **Results:** hba1c in patients with diabetic neuropathy compared to healthy control was statistically highly significant ($p<0.000$). Significant positive correlation was found between serum MDA levels and hba1c ($r = 0.276$, $p < 0.0001$) in patients with diabetic neuropathy. There was statistically significant reduction in the Glutathione peroxidase levels. Further, MDA levels were inversely correlated with GPx ($r = - 0.70$, $p < 0.0001$) levels. **Conclusion and Summary:** oxidative stress is greatly increased in patients suffering from diabetic neuropathy and is inversely related to glycemic control. This may be due to depressed antioxidant enzyme levels and may also be responsible for further depletion of antioxidant enzyme GPx. This worsens the oxidative stress and creates a vicious cycle of imbalance of free radical generation and deficit of antioxidant status in these patients which may lead to nervous system damage causing diabetic neuropathy. A good glycemic control is essential for prevention of diabetic neuropathy.

Keywords: Diabetes, Glutathione Peroxidase, Glycated Hemoglobin, Malondialdehyde

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INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia.[1] Complications such as neuropathy, nephropathy, retinopathy and cardiovascular disorders increase the mortality and morbidity in patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) compared to non-diabetics. Diabetic neuropathy resulting from chronically high blood sugar is one of the most life threatening disorders.[2] In oxidative stress, there is an imbalance between the generations and scavenging of the free radicals, which may be associated with the pathogenesis of the complications of NIDDM including nerve damage leading to diabetic neuropathy.[3] Diabetic neuropathy, can be directly related to oxidative damage and change in antioxidant defenses of nerve cells. Glycated hemoglobin (HbA1C) is a glycoprotein which is used to monitor long term glucose control in people with diabetes mellitus. In addition, glycated hemoglobin is a measure of risk for development of complications of diabetes mellitus. Reduction in 1% HbA1C will decrease long term complication to an extent of 30%.[4] Malondialdehyde (MDA) is a highly toxic product formed in part by lipid peroxide derived free radicals. MDA is widely regarded as a marker of peroxidation damage to cell membranes induced by physical or

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chemical oxidative stress.[5] Glutathione peroxidase (GPx) is biologically important selenium dependent antioxidant enzyme. Insulin deficiency promotes beta oxidation of fatty acids with resulting increase in hydrogen peroxide levels. The paradoxical increase in the level of glutathione peroxidase could be a compensatory mechanism by the body to prevent tissue damage.[6]

MATERIAL AND METHODS

A total of 60 patients in the age group of 40 -80 years attending the OPD and also the inpatients in the JSS Medical College and Hospital, Mysore, were included for the study and the study population was divided into 2 groups . 30 diabetic patients with neuropathy, diagnosed by clinical examination by using diabetic neuropathy examination score and 30 age and sex matched healthy controls. Informed consent was obtained from all the subjects after explaining the nature of study. The study was approved by institutional ethics committee.

Inclusion Criteria

1. Study group: age between 40-80 years, diagnosed with diabetic neuropathy.
2. Control group: age between 40-80 years, without diabetes mellitus.

Exclusion Criteria

1. Age less than 40 years
2. Patients with congestive heart failure
3. Patients with acute and chronic infections
4. Patients with fever
5. Patients with malignancy
6. Patients with acute and chronic nephritis
7. Patients with cirrhosis

Sample Collection:

Five ml of venous blood was collected using aseptic precautions in fasting state and three ml of this was collected in a plain vaccutainer and was analyzed for fasting blood glucose, serum malondialdehyde and the remaining two ml was collected in EDTA vaccutainer for analysis of glycated hemoglobin , serum glutathione peroxidase. Parameters were estimated by following methods,

- Estimation of blood glucose by glucose oxidase method by randox imola.
- Glycated hemoglobin by latex agglutination inhibition method using Toshiba automated analyser
- Serum malondialdehyde by thiobarbituric acid method (TBRAS)[7]
- Glutathione peroxidase by pagelia and valentine method using randox imola an automated analyser[8]

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 enzyme status were correlated with glycemic control in patients with diabetic neuropathy by calculating the Pearson's coefficient of correlation (r value) and the statistical significance was determined from the p value.

OBSERVATION AND RESULTS

Glycosylated hemoglobin levels (HbA1c) was estimated in patients suffering from diabetic neuropathy to assess the glycemic control. Mean level of HbA1C in patients with diabetic neuropathy and healthy controls was 10.17 and 5.14 respectively. Increase in the level of HbA1C in patients with diabetic neuropathy compared to healthy control was statistically highly significant ($p<0.000$). Serum MDA levels were found to be elevated in patients with diabetic neuropathy and the increase was found to be statistically significant. Significant positive correlation was found between serum MDA levels and HbA1c ($r = 0.276$, $p < 0.0001$) in patients with diabetic neuropathy.

Table 1: Mean levels of study parameters in patients with diabetic neuropathy and healthy controls

Parameters	Patients with diabetic neuropathy	Healthy controls
HbA1C	10.17	5.14
MDA(nmol/ml)	7.1	1.81
Glutathione peroxidase (u/l)	2661.27	7604.27

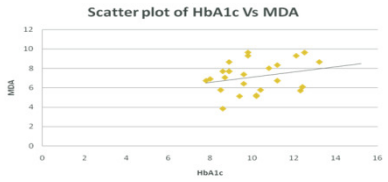

Figure 1: Scatter plot showing correlation between HbA1c and MDA

Figure 2: Scatter plot showing correlation between HbA1c and GPx

DISCUSSION

Diabetic neuropathy is the most common and long term complication of diabetes. There is no single cause for

pathophysiology of diabetic neuropathy.[3] Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes.[9] The oxidation of increased glucose and glycated protiens in diabetes causes production of reactive oxygen species and thus increase oxidative stress.[10,11] The increased levels of ROS like superoxide, hydrogen peroxide, MDA, hydroxyl radical causes oxidation and alteration in the structure o cellular proteins and nucleic acid . This oxidative stress is associated with the development of apoptosis in neurons and supporting glial cells and so could be the unifying mechanism that leads to nervous system damage in diabetes.[12] Neurons not only are lost in diabetes, but their ability to regenerate is also impaired, particularly the small-caliber nerve fibers.[12] In diabetic patients along with the increased production of reactive oxygen species there will be decreased production of antioxidant or effectiveness of antioxidant or both. In our study, there was an increase in the level of MDA, an oxidative stress marker and decrease in the levels of primary antioxidant enzyme GPx and antioxidant vitamin, vitamin C in the peripheral venous blood of type 2 DM patients with neuropathy. Malondialdehyde (MDA) is a highly toxic product formed in part by lipid peroxide derived free radicals. MDA is widely regarded as a marker of peroxidation damage to cell membranes induced by physical or chemical oxidative stress.[5] We found a significant increase in the level of serum MDA in patients who had type 2 DM with neuropathy. GSH is by far the most important antioxidant in most mammalian cell. In particular, the thiol containing moiety of GSH is a potent reducing agent. GSH is maintained at a concentration of 0.2–10mmin all mammalian cells. The most significant role of GSH is as a water-soluble antioxidant. Toxic lipid peroxides combine with two molecules of GSH under the control of GSH peroxidase to form an inert lipid hydroxyl group, GSH disulfide (GSSG), and water. In addition, GSH is involved in amino acid transport, deoxyribonucleotide synthesis, and maintenance of functionally important protein thiol groups in reduced form, and conjugation with toxic compounds such as xenobiotics under the control of glutathione-S-transferase to promote their elimination from the cell. In our study there is a decrease in the level of primary antioxidant enzyme that is glutathione peroxidase and increase in the levels of malonadialdhyde the end product of lipid peroxidation indicating that there is an imbalance between antioxidant and oxidant levels leading nerve damage. The existence of a highly significant inverse correlation between plasma GPx and HbA1C indicates that poor diabetic control is associated with reduced blood antioxidant activity in diabetic neuropathy.

CONCLUSION

The results of the present study suggest that oxidative stress is greatly increased in patients suffering from diabetic neuropathy and is inversely related to glycemic control. This may be due to depressed antioxidant enzyme levels and may also be responsible for further depletion of antioxidant enzyme GPx. This worsens the oxidative stress and creates a vicious cycle of imbalance of free radical generation and deficit of antioxidant status in these patients which may lead to nervous system damage causing diabetic neuropathy. Hence, a good glycemic control is essential for prevention of diabetic neuropathy.

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