Research Article

Evaluation of Oxidative stress markers and antioxidant status in diabetic retinopathy

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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 retinopathy. This study was conducted to evaluate oxidative stress and antioxidant balance in diabeticretinopathy and to correlate this with glycemic control **Method:** Thirty patients with diabetic retinopathy and thirty age matched healthy controls were included in the study. Fasting blood glucose and glycosylated hemoglobin (HbA_{1C}) 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 (GPx) levels were assessed for antioxidant status. **Results:** HbA_{1c} in patients with diabetic retinopathy compared to healthy control was statistically highly significant ($p<0.000$). Significant positive correlation was found between serum MDA levels and HbA_{1c} ($r = 0.387$, $p < 0.0001$) in patients with diabetic retinopathy. There was statistically significant reduction in the Glutathione peroxidase levels. Further, MDA levels were inversely correlated with GPx ($r = - 0.90$, $p < 0.0001$) levels. **Conclusion and summary:** oxidative stress is greatly increased in patients suffering from diabetic retinopathy 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 retinopathy. A good glycemic control is essential for prevention of diabetic retinopathy.

Key words: 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. Hyperglycemia and dyslipidemia in diabetes mellitus induce increased lipid peroxidation and reactive oxygen species formation, an important mechanism in the pathogenesis of micro angiopathy.[2] Oxidative stress is defined as the increased generation of free radicals and/or the impaired compensatory response of endogenous antioxidant defenses, both observed in type 2 diabetes. [3] Oxidative stress is a pathologic condition resulting from either increased production of free radicals or decreased levels of antioxidants. Hyperglycemia, per se or by the promotion of lipid peroxidation of low-density lipoprotein (LDL) can result in the production of free radicals.[4] Glycated hemoglobin (HbA_{1C}) is a glycoprotein which is used to monitor long term glucose control in people with diabetes mellitus. In

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addition, glycated hemoglobin is a measure of risk for development of complications of diabetes mellitus. Reduction in 1% HbA_{1C} will decrease long term complication to an extent of 30%.[5] 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.[6] 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.[7] The present study aims at assessing the role of MDA and suppression of antioxidant defence mechanism of GPxin the development of diabetic retinopathy.

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 retinopathy, diagnosed by H/O of diabetes directand indirect ophthalmoscope examination 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 retinopathy.
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 randoximola.
- Glycated hemoglobin by latex agglutination inhibition method using Toshiba automated analyser
- Serum malondialdehyde by thiobarbituric acid method (TBRAS)[8]
- Glutathione peroxidase by pageliaand valentine method using randoximola an automated analyser[9]

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 (HbA_{1c}) was estimated in patients suffering from diabetic retinopathy to assess the glycemic control. Mean level of HbA_{1C} in patients with diabetic retinopathy and healthy controls was 8.46and 5.14 respectively. Increase in the level of HbA_{1C} in patients with diabetic retinopathy compared to healthy control was statistically highly significant ($p<0.0001$). Serum MDA levels were found to be elevated in patients with diabetic retinopathy and the increase was found to be statistically significant. Significant positive correlation was found between serum MDA levels and HbA_{1c} ($r = 0.832$, $p < 0.0001$) in patients with diabetic retinopathy. GPx level in blood was inversely correlated with HbA_{1c}level and the negative correlation was statistically significant ($r = -0.603$, $p < 0.0001$).

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

Parameters	Patients with diabetic retinopathy	Healthy controls
HbA_{1C}	8.46	5.14
MDA(nmol/ml)	6.27	1.81
Glutathione peroxidase (u/l)	3369.3	7604.27

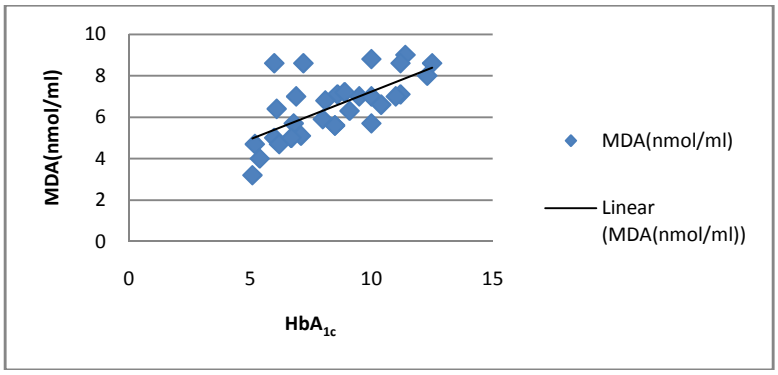

Figure 1: Scatter plot showing correlation between HbA_{1c} and MDA

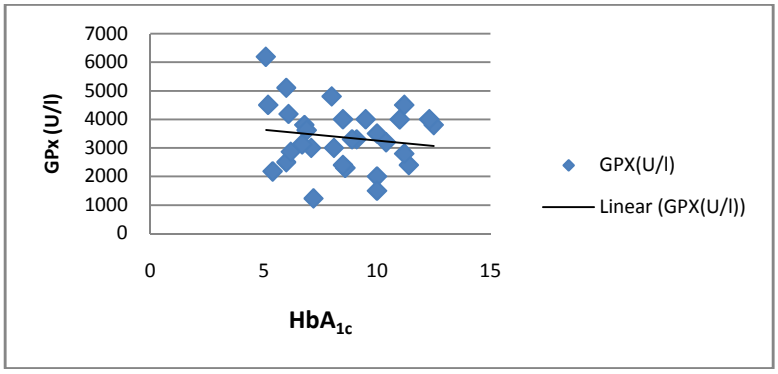

Figure 2: Scatter plot showing correlation between HbA_{1c} and GPx

DISCUSSION

Diabetic retinopathy is a progressive disorder. It is the most common cause of blindness in people aged 30-60 years. With the current trends, after 15 years almost all patients with type1 diabetes and two thirds of those with type 2 diabetes have background retinopathy.[3] The retina has high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders retina more susceptible to oxidative stress.[3]Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes. [10].The oxidation of increased glucose andglycatedprotiens in diabetes causes production of reactive oxygen species and thus increase oxidative stress.[11,12]Reactive O2 metabolites, including free radical,increase with auto-oxidation of glucose and glycosylatedproteins in DM, and with the activation of the sorbitol pathway during hyperglycaemia. Insufficient neutralisation of free radical causes the oxidation of cellular lipids, proteins, nucleic acids, glycolipids and glycoproteins. This oxidative effect also causes damage to the vascular endothelial cells. Additionally,prostaglandin synthesis is affected due to oxidation of arachidonic acid, a polyunsaturated fatty acid. So it is claimed that the long-term damages due to DM are related to the accumulation of increased free radical and LPO products. 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.[13] 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 retinopathy. 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. [6] We found a significant increase in the level of serum MDA in patients who had type 2 DM with retinopathy. 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, 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 malonadialdehyde 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 GPxand HbA_{1C} indicates that poor diabetic control is associated with reduced blood antioxidant activity in diabetic retinopathy.

CONCLUSION

The results of the present study suggest that oxidative stress is greatly increased in patients suffering from diabetic retinopathy. The retina has high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders retina more susceptible to oxidative stress 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 diabeticretinopathy. Hence, a good glycemic control is essential for prevention of diabetic retinopathies.

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