ORIGINAL ARTICLE

IMPACT OF GLYCEMIC CONTROL ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN DIABETIC NEUROPATHY

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ABSTRCAT: INTRODUCTION: Oxidative stress due to enhanced free-radical generation and/or a decrease in antioxidant defense mechanisms has been implicated in the pathogenesis of diabetic neuropathy. This study was conducted to study the impact of glycemic control on oxidative stress and antioxidant balance in diabetic neuropathy. **METHODS:** fifty patients with diabetic neuropathy and fifty age matched healthy controls were included in the study. Glycosylated hemoglobin (HbA1c) was estimated to assess the severity of diabetes and the glycemic control. Serum malondiaaldehyde (MDA) levels were assessed as a marker of lipid peroxidation and hence oxidative stress. Superoxide Dismutase (SOD) levels were assessed for antioxidant status. **RESULTS:** 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, SOD levels were inversely correlated with HbA1c ($r= -0.603$, $p<0.0001$) 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, Superoxide Dismutase, Glycated Hemoglobin, Malondialdehyde.

INTRODUCTION: Diabetic Neuropathy (DN) is a common disorder and is defined as signs and symptoms of peripheral nerve dysfunction in a patient with Diabetes Mellitus (DM) in whom other causes of peripheral nerve dysfunction have been excluded.[1] DM is the most common cause of neuropathy in the Western world. The prevalence of DN varies from 28.5-66 % in western world.[2]In India the prevalence is about 19-60%.[3,4] DN accounts for hospitalization more frequently than any other complications of diabetes and also is the most frequent cause of non-traumatic amputation.

Diabetic autonomic neuropathy accounts for silent myocardial infarction and shortens the lifespan resulting in death in 25-50% patients within 5–10 years of autonomic DN.[5,6] Enhanced oxidative stress and changes in antioxidant capacity are thought to be the etiology of chronic diabetic complications.[7] Hyperglycemia can induce oxidative stress via glucose oxidation and subsequent formation of advanced glycation end products, altered eicosanoid metabolism and decreased antioxidant defenses. Hence the present research was undertaken to study the effect of glycemic control on oxidative stress and antioxidant status in diabetic neuropathy.

METHODS: A total of 100 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. 50 diabetic patients with neuropathy, diagnosed by clinical examination by using diabetic neuropathy examination score and 50 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. Patients with congestive heart failure, acute and chronic infections, fever, malignancy, acute and chronic nephritis and with cirrhosis were excluded from the study.

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 glycatedhemoglobin, serum glutathione peroxidase. Parameters were estimated by followingmethods,

- 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]
- Superoxide Dismutase using RANSOD KIT in the RandoxImola auto analyzer.[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.

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 9.87 and 5.47 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.

Parameters	Diabetic patients with neuropathy	Healthy controls
HbA1c	9.87	5.47
MDA (nmol/ml)	7.14±1.54	1.68±0.43
SOD (u/ml)	110.37±11.49	230.47±42.57

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

Significant positive correlation was found between serum MDA levels and HbA1c ($r = 0.276$, $p<0.0001$) in patients with diabetic neuropathy. SOD level in blood was inversely correlated with HbA1c level and the negative correlation was statistically significant ($r = -0.603$, $p<0.0001$).

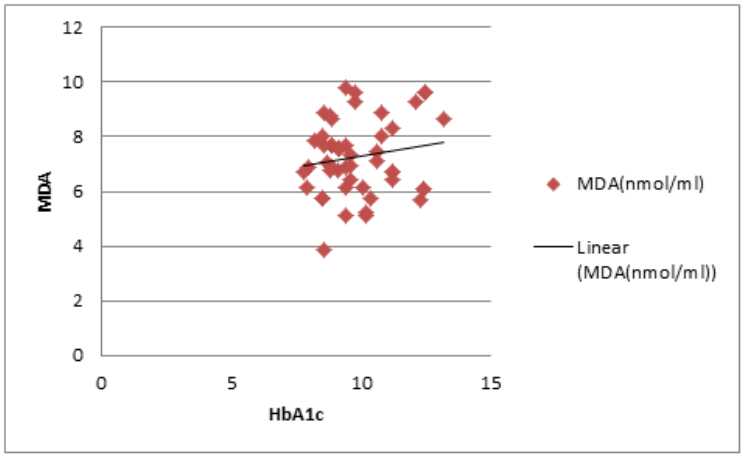

Fig. 1: Scatter plot showing correlation between HbA1c and MDA

DISCUSSION: Diabetic neuropathy is the most common and long term complication of diabetes. Chronic hyperglycemia induces oxidative stress in tissues prone to complications in patients with diabetes.[10] The increased levels of superoxide, hydrogen peroxide, MDA; hydroxyl radical causes oxidation and alteration in the structure of cellular proteins and nucleic acid. This oxidative stress is associated with the apoptosis in neurons and supporting glial cells and so could be the mechanism that leads to nervous system damage in diabetes.[11] Neurons not only are lost in diabetes, but their ability to regenerate is also impaired, particularly the small-caliber nerve fibers.[11]

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 SOD. 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.[12]

We found a significant increase in the level of serum MDA in patients who had type 2 DM with neuropathy. There was a positive correlation between HbA1c and MDA levels in patients with DN. SOD is a specific antioxidant enzyme which detoxifies free radical entities in cells, tissues, and extracellular fluids. The three major isoforms of SOD are: cytosolic CuZn-SOD (SOD1), mitochondrial SOD (SOD2), and extracellular SOD. SOD converts O2 to H2O2 and oxygen. Decreased expression of SOD2 leads to increased oxidative stress. Complete knockout of SOD2 is lethal within days of birth due to renal dysfunction.

Present study revealed that the SOD levels were significantly reduced in subjects with DN compared to healthy controls.in addition there was significant negative correlation between

HbA1c and SOD. The existence of a highly significant inverse correlation between plasma SOD 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 DN 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 SOD. 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 DN. Hence, good glycemic control is essential for prevention of DN.

REFERENCES:

1. Bansal V, Kalita J, Mishra UK. Diabetic Neuropathy. Postgrad Med J 2006; 82: 95–100.
2. Thomas FP. The spectrum of diabetic neuropathy. http://www.neuropathy.org/site/DocServer/Diabetic_Neuropathies.pdf (accessed 19 june 2012).
3. Rani PK, Raman R, Rachapalli SR, Pal SS, Kuldthugam V, Sharma T. Prevalence and risk factors for severity of diabetic neuropathy in type 2 diabetes mellitus. Indian J MedSci 2010; 64 (2): 51-57.
4. Vaz NC, Ferreira AM, Kulkarni MS, Vaz FS, Pinto NR. Prevalence of diabetic complications in rural Goa. Indian J Community Med 2013; 36 (4): 283-6.
5. Levitt NS, Stansberry KB, Wychanck S. Natural progression of autonomic neuropathy and autonomic function tests in a cohort of IDDM. Diabetes Care 1996; 19: 751–4.
6. Rathmann W, Ziegler D, Jahnke M, et al. Mortality in diabetic patients with cardiovascular autonomic neuropathy. Diabet Med 1993; 10: 820–4.
7. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J Biophys 2008; 18 (3): 225-36.
8. Erum SK. Lipid peroxide in cerebrovascular disorderdetermined by a new colorimetric method. Clin Chem Acta 1978; 90: 37-43.
9. Wolliams JA, Wiener G, Anderson PH, McMurray CH. Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep.Res Vet Sci 1983; 34: 253-6.
10. Brownlee M. The pathobiology of diabeticcomplications: a unifying mechanism. Diabetes 2005; 54: 1615–1625.
11. Kumawat M, Singh N, Singh S. Status of antioxidantenzymes and lipid peroxidation in type 2 diabetes mellitus with neuropathy. Annals of Neurosciences 2005; 12 (3): 49-52.
12. Jakus V. The role of free radicals, oxidative stress andantioxidant system in diabetic vascular diseases. Bratesl LekListy 2000; 101 (10): 541-51.

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