ANTIOXIDANT STATUS IN DIABETIC NEUROPATHY

Giriraja Vrushabaiah Kanakapura¹, Pavithra Chandrashekhar Bangalore², Bindumathi Pula Lakshmi³

¹Professor, Department of General Medicine, Sathagiri Institute of Medical Sciences and Research Institute, Bangalore.
²Associate Professor, Department of Oral Pathology and Microbiology, KLE Society’s Institute of Dental College and Hospital, Bangalore.
³Professor and HOD, Department of Internal Medicine, Sathagiri Institute of Medical Sciences and Research Centre, Bangalore.

ABSTRACT

BACKGROUND
Diabetic neuropathy, retinopathy and nephropathy are the chronic complications of diabetes mellitus. Neuropathy, retinopathy and nephropathy are microvascular complication of diabetes mellitus. Antioxidant status is reduced in DM-induced retinopathy and nephropathy. Present study is undertaken to evaluate the degree of oxidative stress in diabetic neuropathy patients.

The aim of the study is to study on oxidative stress as measured by lipid peroxidation marker, malondialdehyde and antioxidant status in type II DM patients with neuropathy and compared them with a controlled nondiabetic group.

MATERIALS AND METHODS
The study included 100 subjects from Sathagiri Medical College, Bangalore, from January 1, 2015, to December 31, 2015, of age group 50 to 70 yrs. out of which 50 patients were non-insulin-dependent DM with neuropathy and rest 50 age and sex matched apparently healthy individuals (control group). Antioxidant status was assessed by measuring superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), Catalase and Reduced Glutathione (GSH).

RESULTS
It showed a significant increase p<0.001 in FBS, PPBS, TC, TG, LDL, VLDL, CAT, MDA, while HDL, GSH, GPX, GR and SOD were found to be decreased significantly (p 0.001).

CONCLUSION
MDA was significantly elevated in diabetic group, whereas antioxidant enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione were significantly decreased, which might be helpful in risk assessment of various complications of DM. The data suggests that alteration in antioxidant status and MDA may help to predict the risk of diabetic neuropathy.

KEYWORDS
Diabetes Mellitus, Antioxidant Enzymes, Diabetic Retinopathy.

HOW TO CITE THIS ARTICLE: Giriraja V, Pavithra C, Bindumathi PL. Antioxidant status in diabetic neuropathy. J. Evid. Based Med. Healthc. 2017; 4(78), 4588-4592. DOI: 10.18410/jebmh/2017/917

BACKGROUND
Diabetic neuropathy is the most common complication of diabetic mellitus affecting as many as 50% of patients with type I and type II diabetics. Diabetic peripheral neuropathy involves the presence of symptoms or signs of peripheral nerve dysfunction in people with diabetes after other possible causes have been excluded.¹ ²

In type I DM, distal polyneuropathy becomes symptomatic after many years of chronic prolonged hyperglycaemia, whereas in type II DM, it may be apparent after only a few years of known poor glycaemic control or even at diagnosis. Symptoms include the following:

- Sensory- Negative or positive, diffuse or focal, usually insidious in onset and showing a stocking and glove distribution in the distal extremities.
- Motor- Distal, proximal muscle weakness, sometimes occurring along with sensory neuropathy (sensory motor neuropathy).² ³
- Autonomic neuropathy that may involve the cardiovascular, gastrointestinal and genitourinary systems and sweat glands.

The two classification systems for diabetic neuropathy are the Thomas system and symmetrical versus asymmetrical system. The Thomas system is as follows:

- Hyperglycaemic neuropathy.
- Generalised systematistical polyneuropathy.
- Sensory neuropathy.
- Sensory motor neuropathy.
- Autonomic neuropathy.
- Focal and multifocal neuropathy.
- Superimposed CIDP.
Distal symmetrical sensory poly motor neuropathy is commonly defined according the following three key criteria.\textsuperscript{3,4,5}

- The patient must have diabetes mellitus consistent with a widely accepted definition.
- Severity of polyneuropathy should be commensurate with duration and severity of DM.
- Other causes of sensory motor polyneuropathy must be excluded.

Asymmetrical neuropathy include the following-

- Carpel tunnel syndrome.
- Other signal or multiple limb mononeuropathies.
- Thoracic radicular neuropathy.
- Lumbosacral radiculoplexus neuropathy.
- Cervical radiculoplexus neuropathy.

The factors leading to the diabetic neuropathy are not understood completely. Multiple hypotheses have been advanced. It is generally accepted to be a multiple factorial process. Development of symptoms depend on many factors, total hyperglycaemia exposure and other risk factors such as elevated lipids, blood pressure, smoking, increased height and high exposure to other potential neurotoxic agents, such as ethanol, genetic factors many also play a role. Important biochemical mechanisms in the development of more common forms of polyneuropathy likely include the polyneuropathy, advanced glycation end products and oxidative stress. Problems that are a consequence of are the co-contributes to these disturbed biochemical process include altered gene expressions with altered cellular phenotypes, changes in cell physiology relating to endoskeleton structure or cellular transport, reduction in neurotrophins and nerve ischaemia.\textsuperscript{5} Clinical trial of best studied neurotrophins, human recombinant nerve growth factor was disappointing. With future refinements, however, pharmacological intervention targeting one or more of these mechanisms may prove successful. In case of focal or asymmetrical diabetic neuropathy syndrome, vascular injury or autoimmunity may play an important role.

Laboratory test that may include the following-

- Fasting plasma glucose.
- Haemoglobin A1c.
- Complete blood count.
- Complete metabolic panel.
- Vitamin B12 and folate levels.
- Thyroid function tests.
- Erythrocyte sedimentation rate.
- Serum protein electrophoresis with immunofixation electrophoresis.
- Antinuclear antibody.
- Rheumatoid factor.
- Paraneoplastic antibodies.
- Anti-SSA and SSB antibodies.
- Rapid plasma regain.
- Genetic screens.
- Haematology screen.

Sequential multiple analysis-7 (renal function and electrolyte imbalances) Complete Metabolic Panel (CMP).

An unifying hypothesis for the pathogenesis of diabetic neuropathy is difficult to synthesise. The heterogeneity in clinical form of diabetic neuropathy illustrates the difficulty in identifying a singular cause. An increasing body of data supports the role of oxidative stress in the pathogenesis of diabetic neuropathy in animal models. Information from clinical studies confirming the role of oxidative stress in the pathogenesis of diabetic neuropathy is limited. However, benefits have been observed with α-lipoic acid, a powerful antioxidant that scavenges hydroxyl, superoxide and peroxyl radicals and regenerates glutathione in many clinical trials.\textsuperscript{6,7,8}

Oxidative stress is defined as the excess formation and/or insufficient removal of highly-reactive molecules (free radicals) such as reactive oxygen species and reactive nitrogen species. It usually occurs when the available supply of the body’s antioxidants is insufficient to handle and neutralise free radicals of different types. The result is massive cell damage that can result in cellular mutations, tissue breakdown and immune compromise.\textsuperscript{9}

There is a high correlation between oxidative stress in diabetes and the development of complications. In type 1 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 glycaemic control. Type 2 diabetic patients have increased lipid peroxidation compared with age-matched control subjects as well as decreased plasma GSH and GSH metabolising enzymes and antioxidant potential, all of which relate directly to the rate of development of complications.\textsuperscript{3,4}

Increase in oxidative stress has clearly been shown to contribute to the pathology of neural and vascular dysfunction in diabetes.

Diabetes-associated oxidative stress is clearly evident in the peripheral nerve, dorsal root and sympathetic ganglia of the peripheral nervous system and endothelial cells; it has implications on nerve blood flow and conduction deficits, impaired neurotropic support, changes in signal transduction and metabolism and morphological abnormalities that are characteristic of peripheral diabetic neuropathy.\textsuperscript{5,10}

Pathogenesis of diabetic neuropathy is complex. Chronic hyperglycaemia is a major factor, which induces nerve fibers injury. Chronic hyperglycaemia causes oxidative stress in tissues prone to complications in patients with diabetes.\textsuperscript{11} High levels of glucose stimulate the polyneuropathy causing osmotic stress, enhance reactive oxygen species generation and play an important role in diabetic angiopathy development.

In recent years, free radicals have assumed an overwhelming importance for the etiopathogenesis of diabetic neuropathy, the most common damaging effect being lipid peroxidation. The self-perpetuating process as a result of hyperglycaemia and dyslipidaemia produces a
number of reacting hydroperoxides and aldehydes leading to microangiopathy and neuropathy in diabetes.\textsuperscript{5,10,12}

Under normal conditions, free radicals are formed in minute quantities and rapidly scavenged by natural cellular defense mechanisms comprising enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione Reductase (GR), Catalase (CAT), etc. An increased production of Malondialdehyde (MDA) has been found in erythrocyte membrane of diabetic patients together with depressed erythrocyte content, i.e. Antioxidant Enzymes (AOEs) and Reduced Glutathione (GSH).

In view of the above consideration, the present study was aimed to evaluate the degree of oxidative stress in diabetic neuropathy subjects.

MATERIALS AND METHODS
The study group included 100 subjects, out of which, 50 patients of DM (females 23 and males 27) with the age group of 50 to 70 years from Sathagiri Medical College from January 1, 2015, to December 31, 2015. These type-2 DM patients with neuropathy were diagnosed on the basis of history, physical examination, nerve conduction study and other biochemical study. Diabetic neuropathy was diagnosed in these patients with the clinical exclusion of renal and cardiovascular involvement. 50 age and sex matched apparently healthy individuals with normal plasma glucose, no neuropathy and with no symptoms suggestive of DM were taken as control.

Under all aseptic conditions 6 mL of venous blood sample was collected from each patient by disposal syringe. This sample was distributed in following vials:

- 0.4 mL in heparinised vial for estimation of plasma Reduced Glutathione (GSH).
- 1.6 mL in citrated vial for estimation of plasma Malondialdehyde (MDA), Catalase (CAT), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx).
- 4 mL in plain vial for estimation of SOD and lipid profile.

Inclusion Criteria
Patients with type II diabetes mellitus between age group 50 to 70 and with neuropathy confirmed clinical and nerve conduction study.

Exclusion Criteria
Patients with acute and chronic inflammatory conditions, other metabolic conditions like ketoacidosis, cerebrovascular accidents or renal diseases as well as smokers, alcoholics and primary hypertensives were excluded from the study.

Antioxidant status was measured by estimating Reduced Glutathione (GSH), Plasma Malondialdehyde (MDA), Catalase (CAT), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx).

All the diabetics were on hypoglycaemic drugs. None of the subjects were on antioxidant supplementation or lipid-lowering drugs.

The study was approved by institutional ethical committee.

Lipid peroxidation product MDA formed a characteristic chromogenic adduct with TBA, which was measured spectrophotometrically after butanol extraction, other AOE and reduced GSH were also analysed. The results were analysed using Student’s t-test.

RESULTS
In type II diabetic subjects suffering from diabetic neuropathy with increase in age, the activity of AOE was found to be decreased significantly. In normal healthy adults, level of Reduced Glutathione (GSH) was 14.53 mg%, in diabetic neuropathy it is 10.14 mg%. The level of Glutathione Peroxidase (GPx) in normal subjects is 8.82 U/g Hb, in diabetic neuropathy it is 8.36 U/g Hb. The level of glutathione reductase in normal group is 16.84 U/g P, in diabetic neuropathy 15.90 U/g P. The level of catalase in normal group is 6 U/g P/mL, in diabetic neuropathy group 8.16 U/g P/mL. Level of superoxide dismutase in normal group is 6.65 U/mg P/mL in diabetic neuropathy group is 3.85 U/mg P/mL. The level malondialdehyde in normal group 4.79 nmol/mL in diabetic neuropathy group 5.64 nmol/mL.

Reduced GSH, GPx, GR and SOD was observed as compared to the control subjects. However, CAT and MAD levels were found to be significantly increased (p <0.001).

In the present study, reduced GSH, GPx, GR, SOD are significantly decreased (p <0.001) table 1 and graph 1 in neuropathy subjects as compared to age and sex matched control group.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>GSH Mg%</th>
<th>GPx U/g Hb</th>
<th>GR U/g P</th>
<th>CAT U/g P/mL</th>
<th>SOD U/mg P/mL</th>
<th>MDA N mol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy adults (n=50)</td>
<td>14.53 ± 2.30</td>
<td>8.82 ± 1.16</td>
<td>16.84 ± 0.56</td>
<td>6.00 ± 0.66</td>
<td>6.65 ± 1.18</td>
<td>4.79 ± 0.72</td>
</tr>
<tr>
<td>Diabetic neuropathy (n=50)</td>
<td>10.14 ± 1.27</td>
<td>8.36 ± 1.18</td>
<td>15.09 ± 0.81</td>
<td>8.16 ± 1.90</td>
<td>3.85 ± 1.07</td>
<td>5.64 ± 0.42</td>
</tr>
</tbody>
</table>

P<0.001, highly significant for GSH, GPx, GR, CAT, SOD, MDA.

Graph Showing the Status of Antioxidant Enzymes and Malondialdehyde (MDA) in Patients with Diabetic Neuropathy

Table Showing the Status of Antioxidant Enzymes and Malondialdehyde in Diabetic Neuropathy Patients
DISCUSSION
It has been postulated that elevated blood viscosity and alteration in the fibrinolytic system occurs in hyperlipidaemia causing diabetic neuropathy. Many biochemical abnormalities have been identified in the nerves in diabetes including elevated oxidative stress, activation of protein kinase C, non-enzymatic glycation and polyneuropathy. However, it is not known, which metabolic abnormalities are critical in the aetiology of diabetic neuropathy. Similar view was expressed in a study conducted by Sadikot et al 2004.2

The nerves are particularly susceptible to oxidative stress because of high combustion of oxygen, its high proportion of polyunsaturated fatty acids. In the present study, reduced GSH, GPx and GR are significantly decreased in neuropathy subjects as compared to age and sex matched control group. In diabetic neuropathy, elevated glucose could increase and NADPH production result in more effective GSH reduction. The decrease in blood GSH levels in diabetic might be in part attributed to the inhibition and inactivation of GR, which is responsible for the regeneration of GSH from its oxidised form by superoxide radical. Hyperglycaemia may cause glycation of GPx, which is responsible for a decreased affinity of an enzyme causing neuropathy.

In this study, the levels of SOD are significantly decreased (p<0.001) in diabetic with retinopathy. Products of lipid membrane per oxidation and other oxidants like H2O2 may react with superoxide dismutase resulting in oxidative modification thereby causing loss of enzyme activity. Also, diabetic hyperglycaemia leads to glycation and inactivation of superoxide dismutase thus attributing to its decrease. Similar results were obtained in a study conducted by Somner, Sanushi et al 2013.5

There is still a controversial view regarding alteration in the activity of catalases in diabetic neuropathy. According to the study, increase in levels of CAT is compensatory for the removal of the free radical in the absence of GPx in type II DM. We found in the study, increase catalase levels, which are in agreement with other studies.

In DM, there is a formation of excessive MDA (lipid peroxides) in cell. There is significantly rise in MDA (lipid peroxides) in this study group (P 0.001). Longer the duration of the disease, the higher are the lipid peroxides levels. Thus, type diabetic neuropathy patients are susceptible to oxidative damage leading to increased lipid peroxidation. Similar results were obtained in a study conducted by Algaidi et al, 2011.10

Hyperglycaemia increases the production of free radicals in our body and damages the tissues like nerves, also lipid peroxidation products are toxic to microvascular cell. The disorders of lipid metabolism and growing intensity of lipid peroxidation caused by diabetes maybe one of the factors to develop neuropathy.

Hyperglycaemia causes increased levels of glucose in nerves leading to saturation of normal glycolytic pathway. Extra glucose is shunted into the polyneuropathy and converted to sorbitol and fructose by the enzyme aldose reductase and sorbitol dehydrogenases. Accumulation of sorbitol and fructose leads to reduced nerve myo-inositol, decreased membrane Na+/K+ ATPase activity, impaired axonal transport and structural breakdown of nerves causing abnormal action potential propagation. This is rationale for the use of aldose reductase inhibitors to improve nerve conduction. Similar hypothesis was expressed in a study conducted by Russel et al 2002.7

The nonenzymatic reaction of excess glucose with proteins, nucleotides and lipids results in Advanced Glycation End-Products (AGEs) that may have a role in disrupting neuronal integrity and repair mechanisms through interference with nerve cell metabolism and axonal transport. Similar view was expressed in a study conducted by Evans et al 2002.9

The increased production of free radicals in diabetes may be detrimental via several mechanisms that are not fully understood. These include direct damage of blood vessels leading to nerve ischaemia and facilitation of AGE reactions. Despite the incomplete understanding of these processes, use of the antioxidant alpha lipoic acid may hold promise for improving neuropathic symptoms. Similar view was expressed by Kiritoshi et al 2003.6

Problems that are a consequence of or co-contributors to these disturbed biochemical processes included altered gene expression with altered cellular phenotypes, changes in cell physiology relating to endoskeleton structure or cellular transport, reduction in neurotrophins and nerve ischaemia. Clinical trials of the best studied neurotrophin, human recombinant nerve growth factor were disappointing. With future refinements, targeting one or more of these mechanisms may prove successful.

In the case of focal or asymmetrical diabetic neuropathy syndromes, vascular injury or autoimmunity may play more important roles.10,11

Both dyslipidaemia and lipid peroxidation also contribute to oxidative stress. Metabolically, active Free Fatty Acids (FFAs) exert an inhibitory effect on the adenosine nucleotide translocation with a resultant decrease in available ADP resulting in slowing down of the flow of electrons anions (O2) resulting in oxidative mitochondrial stress. Advanced glycation products bind to retinal pigment endothelial cell initiating wide range of cellular events leading to retinopathy. Apart from hyperglycaemia and dyslipidaemia, the weakness of the antioxidant defense systems maybe the biochemical background for the pathogenesis of endothelial dysfunction associated with diabetes mellitus and lead to microvascular damage resulting in diabetic neuropathy. Similar view was expressed by Zatalia et al 2013.5

CONCLUSION
In our study on oxidative stress as measured by lipid peroxidation marker, malondialdehyde and antioxidant status in type 2 DM patients with or without neuropathy and compared them with a controlled nondiabetic group, MDA was significantly elevated in both the diabetic groups, whereas antioxidant enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione were significantly decreased, which might be helpful in risk assessment of various complications of DM.
The data suggests alteration in antioxidant status and MDA may help to predict the risk of diabetic retinopathy.

REFERENCES