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Balance between antioxidant enzyme and lipid peroxidation in diabetes neuropathy

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Abstract
The study was designed to compare the balance between lipid peroxidation and antioxidant enzyme activity in Type 2 diabetes neuropathy patients. The result was compared with the healthy control in the same population. Diabetic neuropathy (DN) patients had higher antioxidant and malondialdehyde (MDA) level than control subject. The study was conducted in the out patients department of endocrinology VIMS, Ramakrishna Mission Seva Pratishthan, Kolkata. The study involved 68 individuals, of which 38 patients with type 2 diabetic neuropathy (confirmed positive sensory/polyneuropathy problem by nerve conduction velocity test) and 30 individual without a history of diabetes as a control group. All procedure was done with the consent of participants. The result showed a positive correlation between superoxide dismutase (SOD) and lipid peroxides (LP) level in diabetic neuropathy patients. These data suggests that MDA, as a lipid peroxides indicator, is higher in diabetic neuropathy patients probably due to chronic high blood sugar followed by higher oxidative stress. It has also been suggested that increased SOD level in diabetic neuropathy (DN) group appeared as a result of protective and adaptive mechanism developed against oxidative stress i.e. free radical generation due to hyperglycemia.

Keywords: diabetes mellitus, diabetes neuropathy, superoxide dismutase, lipid peroxidation, malondialdehyde

Introduction
There is considerable evidence that hyperglycemia represents the main cause of complications of diabetes mellitus (DM), and oxidative stress resulting from increased generation of reactive oxygen species plays a crucial role in their pathogenesis. Under chronic hyperglycemia state, oxidative stress develops in diabetes due to poor elimination of reactive oxygen species (ROS) from different tissue (Basta *et al* 2002, Kowluru and Chan 2007) [1, 2]. In fact, in the absence of an appropriate response from endogenous antioxidant mechanisms, the redox imbalance causes the activation of stress-sensitive intracellular signalling pathways. The latter plays a key role in the development of late complications of DM, as well as in impaired insulin secretion. Oxidative stress has been considered to be a pathogenic factor of diabetic complications including nephropathy. There are many controversies and limited studies regarding the antioxidant enzymes in diabetic nephropathy. However, there are reports that in diabetes, the disturbed equilibrium between prooxidants and antioxidants alters the metabolic status of body leading to the development of micro vascular complications like neuropathy (Kumawat 2009) [3]. Lipid peroxidation, owing to free radical activity, plays an important role in the development of complications of diabetes. Although increased level of lipid peroxidation as a consequence of free radical activity, have been reported in both Type 1 and Type 2 diabetes with vascular complications (Jennings *et al* 1991, Griesmaxcher *et al* 1995) [4] other studies failed to detect any significant elevation in lipid peroxidation in diabetic patients, probably owing to heterogeneity of patient's population (Velazqez *et al* 1991). The occurrence of free radical induced lipid peroxidation causes considerable changes in the cell membrane (Agrawl *et al* 1985, Surjawanshi 2006). Peroxidation of lipid membrane has been related to the pathogenesis of many degenerative damage to DNA. Thus lipid peroxide as well as antioxidant activity in the blood provide useful information for the prognosis of diabetes in which secondary disorders are often fatal.

Method
The study was conducted at the genetics department of Vivekananda Institute of Medical Sciences (VIMS), Ramakishna Mission Seva Pratishthan (RKMSP), Kolkata and was approved by the institutional Ethics Committee. The study group consist of type 2 diabetes patients and healthy control was taken from the outpatient Department of Endocrinology, VIMS, RKMSP, Kolkata. For assessment of Diabetic Neuropathy (DN) presence according to clinical symptom, duration medication and socioeconomic condition, a questionnaire was used. All procedure was done with the consent of participant. The study involved 68 individuals and consisted of 38 patients with type 2DN along with neuropathy

and 30 patients without a history of diabetes as a control group. The diabetic neuropathy patients were included in this study after confirmation gained by positive nerve conduction velocity (NCV) report and impaired fasting glucose test (>126 mg/dl).

For superoxide dismutase (SOD) and lipid peroxides (LP) activity 3 ml of venous blood was collected from cubital vein in EDTA (K2) containing vial. After collection, the blood was kept 30 min for settling down the cells. Then the whole blood was centrifuged at 3000 rpm for 10 min to separate the RBC and plasma. RBC was taken for the assessment of SOD activity and Plasma for lipid peroxidation.

SOD activity in RBC: The collected RBC was washed with 0.9% NaCl solution and centrifuged for 10 min at 3000 rpm after each wash (2 times), The RBC was ruptured by chilled water. The lysate was diluted with 0.01 m/L phosphate buffer (pH 7.0). The SOD activity was evaluated by spectrophotometric method (505 nm) [Spectra Max UV-Visible spectrophotometer (SPECORD)] (Woolliams 1983) [9].

Determination of Lipid Peroxide: Lipid peroxidation was measured by quantifying malondehydrate (MDH) in blood samples (plasma) of patients following thiobarbituric acid-reactive substances (TBARS) assay. The lipoproteins of blood plasma were precipitated by addition of 0.05(M) trichloroacetic acid (TCA) and 0.67 % thiobarbituric acid (TBA) to the sample. The union of lipid peroxide and TBA was carried out by heating in a boiling water bath for 60 min. The mixture was cooled in room temperature and resulting chromogen was separated by centrifugation. The colour supernatant was measured at 532 nm (Satoh 1978) [10]. Concentration is calculated with an extinction coefficient ∑M=155 mM-1cm-1 and result are presenting as nM/ml.

Table 1: Age and Sex distribution of Diabetic Neuropathy patients.

Age (Years)	Sex		Total	Percentage (%)
	Male	Female		
Below 50	6	11	17	44.73
Above 51	15	6	21	55.26
Total	21	17	38	100

Result

All the 38 patient age ranging from 27 years to 70 years were selected for the present study and were type 2 diabetes neuropathy as confirmed by nerve conductive velocity test. Among the study group, 44.73 % patients were below 50 years of age and 55.26 % were above 51 years of age (Table 1). The duration of disease of the patients was ranging from 0 days to 20 years.

The antioxidant status as evidenced by erythrocyte SOD level was found to be increased significantly in relation to that of control group. (Fig 1).

It is evident from the results that lipid peroxide was significantly increased as reflected in increase malondialdehyde (MDA) level in diabetic neuropathy when compared with that of healthy control (Fig 3).

It is to be noted that an interesting observations has been resulted from the present study, the SOD activity and MDA level were increased significantly in male group with the increasing age. No such observation was noted in case of female group. (Fig 2 & 4).

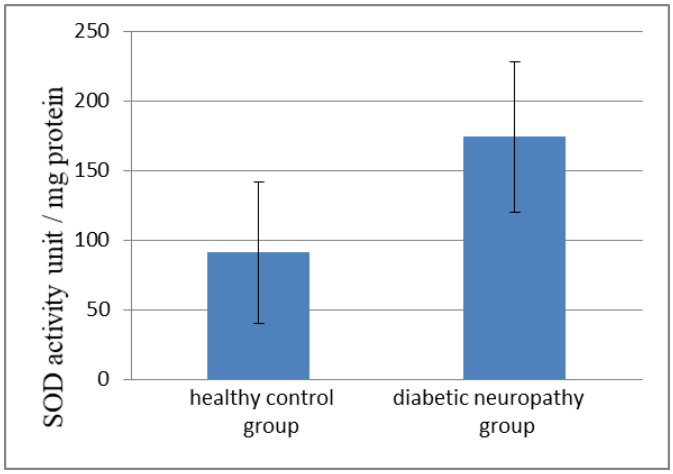

Fig 1: SOD activity of healthy control group and diabetic neuropathy group.

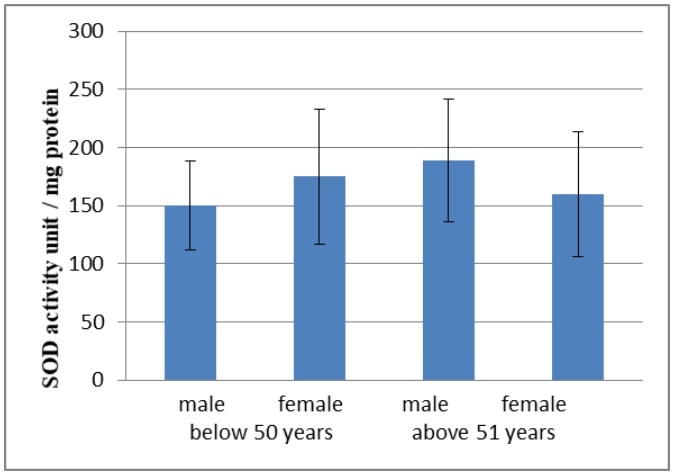

Fig 2: SOD activity of diabetic neuropathy group

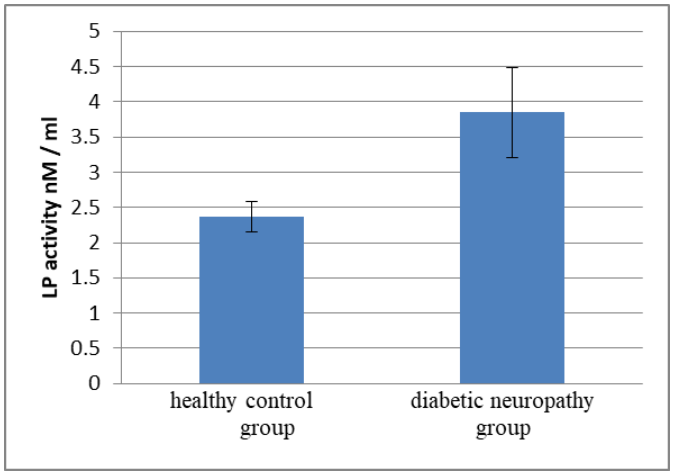

Fig 3: lipid peroxide activity of healthy control group and diabetic neuropathy group.

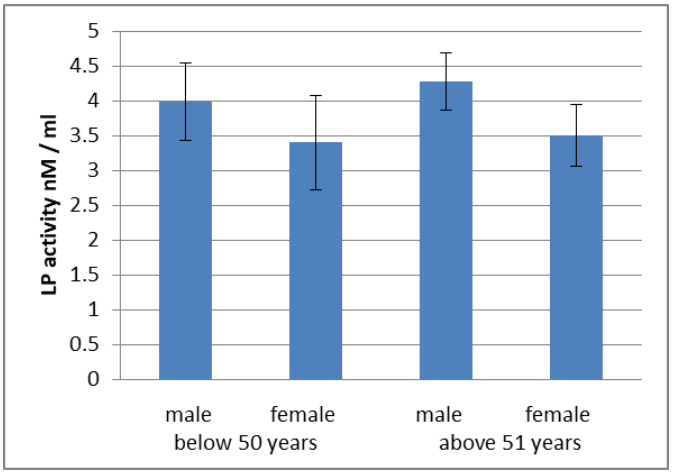

Fig 4: LP activity of diabetic neuropathy group.

Discussion

Diabetes mellitus has been known to be a state of excess generation of free radicals contributed by several mechanisms which mainly included hyperglycemia and antioxidant status resulting oxidative stress. This oxidative stress promot the development and progress of diabetes and its complications. Persistant hyperglycemia in diabetes mellitus has been reported to induce autooxidation of glucose generating oxidative stress (Agarwal *et al*. 1985) [7] which ultimately disturbs the antioxidant defence system of the cell by increasing the functional activity of superoxide anion (O^{2-}), the hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2). However excessive production of free radicals in diabetes results in damaging cellular proteins and membrane lipids (Bonnetout *et al* 2000). It has also been shown that there is increased lipid peroxidation owing to elevated free radicals in both type 1 and type 2 diabetes. (Griesmacher *et al* 1995, Kalavanam *et al* 2010) [5].

In the present study we have evaluated the importance of SOD (antioxidant) activity and lipid peroxidation in diabetes neuropathy. The study showed significant increase of lipid peroxidation as reflected in MDA level. MDA level and SOD activity in diabetic neuropathy compared to healthy individual and as such it indicates a marked oxidative stress resulted in diabetes neuropathy. Several different mechanisms have proposed in favour of increased oxidative stress in diabetes. Hyperglycemia in diabetes may increase ROS production and alter the balance of oxidant and antioxidant activity and some of these mechanism operate simultaneously in a synergistic fashion (West 2000) [13].

The present data shows a positive correlation between glucose concentration and superoxide dismutase. The results corroborate with other investigators where in it has been observed that SOD activity increased in diabetes when compared with that of control without diabetes (Thomas 2014) [14]. The increased SOD activity in diabetic neuropathy may counteract the deleterious effects of oxidative stress helping to prevent many complications associated with diabetes (Maryani 2005). Lipid peroxidation is a free radical related process, which is potentially harmful because its uncontrolled, self enhancing process causes disruption of membrane lipids and other cell components. Diabetes produces disturbances of lipid profiles especially an increased susceptibility to lipid peroxidation (Giugliano *et al* 1996) as indicated by high free radical products (Seghrouchni *et al* 2002) [17]. To encounter the effect of free radicals, the body is endowed with another category of compounds called antioxidants. These antioxidants are supposed to protect/prevent/delay the oxidative destruction of biomolecule (Halliwell 1990) [18]. Increased free radical production is said to mediate tissue injury in a wide range of disease and diabetes mellitus is no exception (Richards 1990, Yilgiz *et al* 2002) [19].

So the integrated antioxidant system acts synergistically to protect tissue against free radical attack and onset of disease. The relationship between hyperglycemia and oxygen free radical is supported by the present results demonstrating an association between blood levels of glucose and enzymatic antioxidant like superoxide dismutase. The increase SOD activity culminated as a result of stress produce by high concentration of free radicals due to hyperglycemia. The present results are in accordance to the study performed by others (Bandeira *et al* 2012) [21]. It has been suggested that increased SOD level in diabetic neuropathy group may appear as a result of a protective and adaptive mechanism developing in the tissue, may also be an indicator of the increased free radical generation in diabetes (Tomas 2014). On the contrary, there are some studies, in which they have shown a decrease SOD level in diabetes (Ziegler D, *et al* 2004, Piwowar A *et al* 2007, Kasznicki J, *et al* 2012) [22, 23, 24]. Our results are in agreement with Moussa who indicated an increased SOD level in type I and Type II diabetes.

Increased lipid peroxide may be due to the increased glycation of protein in diabetes mellitus. The glycated protein might themselves act as a source of free radicals. Lipid peroxide formed stimulate the cyclooxygenase and prostaglandin and thromoboxane synthesis. This will cause increase platelets aggregation leading to vascular complications. The results depict a clear association between lipid peroxidasion and glucose concentration which may also be thought to play a role in increased lipid peroxidation in diabetes mellitus (Suryawanshi 2006) [8].

More over a significant difference was revealed in male patients when age was considered. No such observation was noted in case of female. It is suggested that number of sample needs to be increased to justify this variation. However statistically significant difference confirms that there is an increased oxidative stress in diabetes compared to non diabetes counterparts and emphasizes the importance of assessing their markers for early diagnosis and therapeutic intervention.

However it is worth mentioning that experimental evidence indicating that over expression of antioxidant enzyme can protect neurons against oxidative injury are still lacking (Negi *et al* 2011) [25]. This study encountered with a limited sample size. The further study requires strategies for exploring this variables with a larger sample size with more sensitive indicator in diabetic neuropathy patients.

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