STATUS OF ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN TYPE 2 DIABETES MELLITUS WITH NEUROPATHY

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Abstract

Background and Objectives : In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen free radicals. Hyperglycemia could induce oxidative stress and become the main factor for predisposing the complications in diabetes. Diabetic neuropathy can be directly related to oxidative damage and change in antioxidant defences of nerve cell. Oxidative stress appears to be primarily due to nerve ischemia and hyperglycemia auto oxidation.

Aim of Study : The study is being aimed to find out the status of lipid peroxidation product i.e. malondialdehyde (MDA) and antioxidant enzymes (AOEs) such as glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) which might be helpful in risk assessment in subjects suffering from cerebrovascular stroke.

Methodology : The study included 125 subjects (50-70 yrs), out of which 50 patients are non insulin dependent diabetes mellitus (NIDDM) with cerebrovascular stroke and 75 age matched healthy control. The status of fasting blood sugar (FBS), reduced glutathione (GSH), GPx, GR, CAT, SOD and MDA were determined.

Results : Our results showed highly significant increase ($P < 0.001$) in FBS, CAT and MDA while GSH, GPx, GR and SOD were decreased significantly ($P < 0.001$).

Conclusion : The data suggest that alterations in antioxidants status and MDA may help to predict the risk of diabetic neuropathy.

Key Words : Diabetes mellitus, Neuropathy, Antioxidant enzymes

Malondialdehyde, Oxidative stress.

Introduction

In NIDDM, the insulin secretion is normal in amount but usually the target cells of insulin are, due to one reason or other, resistant to insulin. In some cases, there is deficiency of insulin receptors, but in most cases, the site of resistance is beyond the receptor. Hyperglycemia, the primary clinical manifestation of diabetes, has been accepted as being essential for the development of diabetic neuropathic complications. Many evidence have indicated that some biochemical pathways strictly associated with hyperglycemia (non enzymatic glycosylation, glucose auto oxidation, polyol pathways) can increase the production of free radicals (1).

In patients with type 2 diabetes, previous prospective studies have shown an association between the degree of hyperglycemia and increased risk of micro vascular complications (2,3). Diabetic neuropathy can be directly related to oxidative damage and change in antioxidant defences of nerve cell. Polyneuropathy is the most common form of diabetic neuropathy and presents distal symmetric sensory loss, hyperaesthesias and pain. Physical examination reveals sensory loss, loss of ankle reflexes and abnormal position sense. Oxidative stress appears to be primarily due to nerve ischemia and hyperglycemia and auto oxidation. Peripheral nerve tissue has a lower GSH content and lower activities of antioxidant enzymes (4). When tissues are crushed or torn, iron can be released from cells, both as free iron and in the form of haem-containing proteins (such as myoglobin) and as storage protein ferritin. Free iron can convert O2.- and H2O2 into highly damaging OH and O2 can cause a limited release of iron from ferritin and H2O2 can release iron from haem proteins. Haem proteins can accelerate lipid peroxidation, since the iron content of human tissues increases with age, perhaps iron is mobilized in greater amounts as a result of injury and free radical damage becomes more severe in injured older tissues. The brain tissue is very rich in polyunsaturated fatty acid side chains, the level of antioxidant defence enzymes in the brain is moderate. The cerebrospinal fluid (CSF) has little transferrin and so cannot bind with released iron. Iron ions are proved to be generating free radicals. Free radical reactions probably occur in damaged brain tissue after injury (e.g. that caused by a blow to the head). It has been suggested that these reactions deteriorate the consequences of initial injury to the brain or spinal cord, by spreading the damage into surrounding areas. Similar free radical reactions may occur after stroke, spreading the damage beyond the ischaemic area. In stroke, a blood clot forms in a artery in the brain shutting of the blood supply to part of this organ. Deprivation of blood flow, and the resulting deprivation of O2 cause many metabolic changes. Ischaemic tissues become more acidic (their pH falls). ATP levels drops and iron ions are released from storage sites. Mitochondria may be disrupted. When a tissue is ischaemic., the O2 supply must be restored as fast as possible before the cells die. Re oxygenation of badly damaged tissue can wash metal ions and other enzymes into the blood stream and can cause damage to blood cells. Under normal conditions, free radicals superoxide anion (O2-.), the hydroxyl radical (OH-.) & hydrogen peroxide (H2O2) are formed in minute quantities and are rapidly scavenged by natural cellular defense mechanisms mainly enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) etc. The RBC were selected for the estimation of these enzymes because they are easily accessible rich in thiol functions and are potentially involved in attack from the protection against free radicals (5). An increased production of malondialdehyde (MDA), a marker for lipid peroxidation has been found in erythrocytes

membrane of diabetic patients together with depressed erythrocyte content that is antioxidant enzymes (AOEs) and reduced glutathione (GSH) (6).

Material & Methods

The present study was carried out in 125 subjects (50-70 yrs), out of which 50 patients are NIDDM with cerebro vascular stroke and 75 age matched healthy control. We took all patients from the Medical wards/ICU/OPD of Department of neurology, JA group of hospital, Gwalior, under the supervision of medical officer and the written consent is also taken from patient before analysis of blood. All ethical norms were followed during study. The analysis was done in the department of Biochemistry, G.R. Medical College, Gwalior. The 5 ml blood sample was collected in fasting condition for the analysis of various parameters :

1. Fasting blood sugar (FBS) is estimated by method of GOD-POD Trinder P (7).
2. Glutathione (GSH) is estimated by method of Beutler E. et al (8).
3. Glutathione peroxidase (GPx) is estimated by method of Hafeman DG et al (9).
4. Glutathione reductase (GR) is estimated by method of Horn HD (10).
5. Catalase (CAT) is estimated by method of Sinha KA (11).
6. Superoxide dismutase (SOD) is estimated by method of Mishra HP et al (12).
7. Plasma malondialdehyde (MDA) is estimated by method of Jean CD et al (13).

The statistical analysis was done by Student's 't' test.

OBSERVATIONS

Table No. 1. Showing the status of study parameters in normal healthy control (50-70yrs.)

n=75	FBS mmol/l	GSH mg%	GPx U/gmHb	GR U/gmP	CAT U/gmP/ml	SOD U/mgP/ml	MD Anmol/ml
MIN	3.01	10.0	6.2	16.0	4.9	4.3	3.0
MAX	6.64	18.0	11.0	18.3	7.0	8.9	6.0
MEAN	4.84	14.33	8.66	16.67	5.96	6.60	4.99
±S.D	±0.65	±2.15	±1.09	±0.52	±0.68	±1.11	±0.76
S.E	0.07	0.24	0.12	0.05	0.07	0.12	0.08

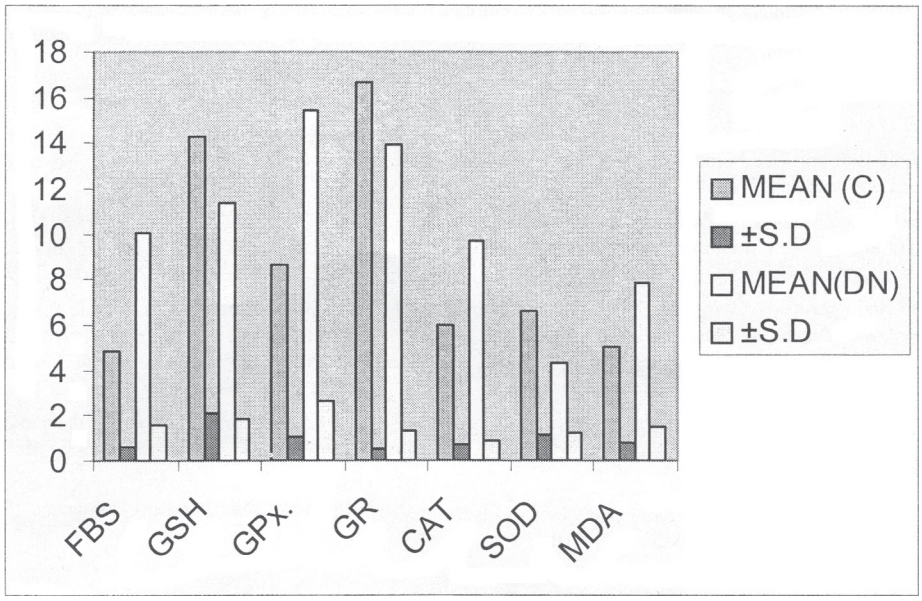
Table No. 2. Showing the significant changes in all study parameters in diabetic subjects (50-70yrs) suffering from cerebrovascular stroke as compared to age matched healthy control.

N=50	FBS mmol/l	GSH mg%	GPx U/gmHb	GR U/gmP	CAT U/gmP/ml	SOD U/mgP/ml	MDA nmol/ml
MIN	7.01	8.24	10.51	12.0	8.3	2.24	5.38
MAX	12.81	14.63	20.0	16.8	11.61	6.9	10.63
MEAN	10.10	11.42	15.44	13.93	9.74	4.36	7.85
±S.D	±1.61***	±1.83***	±2.65***	±1.32***	±0.92***	±1.23***	±1.50***
S.E	0.23	0.35	0.39	0.18	0.13	0.21	0.23

*** (P<0.001) Highly Significant
Results are expressed as Mean±S.D

Results & Discussion

In Type 2 diabetic subjects suffering from cerebrovascular stroke, the activity of antioxidant enzymes (AOE) altered significantly (14,15). The same observations present in our study indicating significant decrease (P< 0.001) of GSH, GR and SOD and significant increase (P< 0.001) in GPx, CAT as compared to age matched control subjects (Table No.1.). The lipid peroxide malondialdehyde (MDA) also found significantly increased (P< 0.001). In our study we have used erythrocytes as a model to study oxidative stress (OS). It is a target for oxidative reaction because of high oxygen tension and the presence of hemoglobin and plasma membrane rich in polyunsaturated fatty acid (PUFA). The mean value of SOD, GR are significantly decreased (P< 0.001) as compared to control group and the mean value of GPx and catalase were found significantly increased (P< 0.001) as compared to control group. The values of MDA was also significantly increased in diseased group (Table No 2, Graph I).



Graph I showing the significant changes in all study parameters in diabetic subjects (50-70yrs) suffering from cerebrovascular stroke compared to age matched healthy control.

All diabetic patients had severe hyperglycemia. These observations indicates a chronic oxidative stress in diabetic group.

The level of glutathione reductase (GR) in erythrocytes is significantly decreased ($P < 0.001$). This decrease may be due to glycation of enzyme (16) and reduction in NADPH which is used by GR as a source of reducing power due to hyperglycemia. The autooxidation of glucose results in the formation of hydrogen peroxide (H2O2) which inactivate SOD (17). The activity of SOD decreases in erythrocytes also due to ageing or increase in the glycation of SOD (18). Hydrogen peroxide is removed by the enzymatic activities of catalase and GPx. This study is consistent with the observation of Ceballos et al 1998 (19) who explained that during ageing process the steady state concentration of erythrocytic H2O2 may be much higher which could lead to enhance peroxidation of PUFA present in cell membrane. This enhanced lipid peroxidation leads to the lysis of erythrocytes resulting in the increase in the level of antioxidant enzymes GPx and catalases. Increased level of MDA observed in neuropathic group is consistent with the findings of Nohl etal 1991 (20). Peroxidation of lipids in fatty acids may lead to a radical chain reaction and because of this one substrate radical (R.) may result in the formation of many equivalents of lipid peroxides (.LOOH). GSH levels are known to decline with age. Plasma contains a significant level of glutathione, mainly in reduced form. Since clearance of plasma GSH is very rapid, the plasma levels reflect hepatic GSH output. Under normal condition of steady state, to maintain homeostasis, the hepatic pool of GSH is depleted at same rate as it is repleted. This dynamic equilibrium therefore sustained by a continuous turnover (synthesis-degradation) of hepatic GSH. The decrease in the GSH levels, with ageing and during disease conditions may be attributed to the fact that GSH is easily and rapidly converted to oxidized glutathione (GSSG) due to oxidation of its thiol group (-SH) by oxiradicals associated with ageing as well as pathological conditions. The rate of oxidative conversion of GSH into GSSG is very high in comparison to the rate of its repletion. This phenomenon results in the depletion of the levels of GSH during ageing and diseased states (21). In diabetes with cerebrovascular stroke, the patients have oxidative stress due to derangement of metabolism and change in levels of AOEs. The neurological damage associated with diabetes may be due to the above quoted status of antioxidant enzyme system.

Conclusion

In diabetic subjects oxidative stress is significantly higher in the studied diseased condition. Activities of antioxidant defence enzymes indicate detrimental alterations in comparision to that observed in control group. The oxidative stress peaks up in complex disease conditions of neuro disorders inflicted by diabetes and strokes. Hence the clinical use of the antioxidants at the appropriate stage of the disease may prevent, delay or reverse the multi organ complications. Researchers from developed countries have also recommended for the same.

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