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A COMPARATIVE STUDY ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN ISCHEMIC STROKE PATIENTS WITH AND WITHOUT DIABETES

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ABSTRACT

The study was conducted to find out the extent of lipid peroxidation and antioxidant status in ischemic stroke patients (ISPs) with and without diabetes. Malondialdehyde (MDA) was studied as a marker of lipid peroxidation. Glutathione (GSH), uric acid and ceruloplasmin were estimated to study the antioxidant potential of ISPs. Significantly higher levels of MDA were found in both the groups of ISPs and the increase in MDA was more in ISPs without diabetes. GSH levels were decreased significantly in both the groups of ISPs and maximum decline was found in ISPs with diabetes. Uric acid levels were significantly increased in both the groups of ISPs. Ceruloplasmin levels were increased significantly in ISPs without diabetes, whereas its levels were slightly decreased in ISPs with diabetes. A negative correlation was found between MDA and the antioxidants GSH, uric acid and ceruloplasmin in ISPs with diabetes. This study suggests that there is an association between ischemic stroke and increased oxidative stress and the antioxidant potential is impaired in both the groups of ISPs with and without diabetes.

KEY WORDS

Ischemic stroke , Lipid peroxidation, Antioxidants.

INTRODUCTION

Stroke is characterized by the sudden loss of circulation to an area of the brain resulting in a corresponding loss of neurologic function. Stroke is the third most common cause of death and is a major cause of morbidity particularly in the middle aged and elderly population (1,2). Central nervous system (CNS) damage occurs in stroke as a result of hypoxia. Strokes are currently classified as either hemorrhagic or ischemic. Ischemic stroke occurs when the blood supply to a part of the brain is suddenly interrupted by occlusion. Hemorrhagic stroke occurs when a blood vessel in the brain bursts, spilling blood into the spaces surrounding the brain cells or when a cerebral aneurysm ruptures. People most at risk for stroke are older adults, particularly those with high blood pressure, who are sedentary, overweight ,smoke or have diabetes.

Diabetes is a strong risk factor for Ischemic stroke, perhaps because of accompanying risk factors, such as obesity and high blood pressure (3). Studies have also implicated insulin resistance, which usually occurs in people with non-insulin dependent diabetes as an independent factor in the development of atherosclerosis and stroke.

Ischemia causes a cascade of metabolic events that eventually lead to neuronal damage and death. Neuronal nitric oxide synthase produces nitric oxide which is able to react with superoxide generating the highly reactive radical peroxynitrite (4,5). Experimental studies provide evidence of an association between ischemic stroke and increased oxidative stress, but data in humans are still limited and controversial . We have particularly studied the products of lipid peroxidation since the brain is very rich in polyunsaturated fatty acids which are highly susceptible to free radical attack (6-9). The present study was therefore undertaken to compare the oxidative stress and the antioxidant status in ischemic stroke patients with and without diabetes .

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MATERIALS AND METHODS

The study group consisted of 30 ischemic stroke patients between the age group of 40-86 years of both sexes (22 males and 8 females). These patients were admitted to Kasturba Medical college Hospital, Attavar, Mangalore or Shaffi Ayurvedic clinic , Mangalore with a diagnosis of ischemic stroke of >24 h duration . Computerized Tomography of the head was done in all the patients which showed cerebral infarction after the onset of stroke. These patients were divided into two groups. 1. Ischemic stroke patients with diabetes (15 patients). 2. Ischemic stroke patients without diabetes (15 patients). Persons who were smokers, alcoholics, renal failure, gout, arthritis and severe medical or psychiatric illness were excluded from the study. The control group consisted of 20 healthy individuals of the same age group of both the sexes (17 males and 3 females). Random venous blood samples were collected in EDTA bottles and in bottles without any anticoagulant from ischemic stroke patients and normal healthy individuals. 0.2 ml of the whole blood was used for Glutathione (G-SH) estimation . Blood collected without anticoagulant was centrifuged at 3000g for 10 minutes. Serum was collected carefully and used for estimation of Uric acid and Ceruloplasmin. Blood collected in EDTA was also centrifuged at 3000g for 10 minutes. The separated cells were washed thrice with 0.9% cold saline. The RBCs were then suspended in an equal volume of 0.9% saline and used for the estimation of Malondialdehyde(MDA).

Lipid Peroxidation(MDA) : The susceptibility of erythrocytes to undergo oxidation in the presence of 0.44 mol/L hydrogen peroxide was assessed. Lipid peroxidation was studied as Thiobarbituric acid (TBA) reaction products. The method of Stocks and Dormandy was followed with certain modifications (10). Values were expressed as Nanomoles of MDA formed per deciliter, taking the molar absorption of MDA as $1.5x10^5$.

Glutathione was estimated by the method of Beutler et al (11). Ceruloplasmin was estimated using the method of Sunderman et al.(12). Uric acid was estimated by Uricase method of Kabasakalian et al (l3).

Statistical Analysis : Statistical analysis was done by using Kruskal – Wallis test and Mann Whitney U- test for the comparison of different parameters. Chi square text is also being done for qualitative data. Correlation between the variables were estimated by Pearson's Correlation coefficients.

RESULTS

The increase in MDA in ischemic stroke patients(ISPs) is highly significant (<0.001) when compared to control subjects. This indicates that lipid peroxidation is significantly increased in ISPs and the increase in MDA is more in ISPs without diabetes ($p<0.001$). The GSH levels are decreased significantly in ISPs compared to control subjects($p<0.001$). Maximum decline in GSH is found in ISPs with diabetes($p<0.001$). The Ceruloplasmin levels are increased significantly ($p<0.05$) in ISPs without diabetes when compared to control subjects, whereas its levels are slightly decreased in ISPs with diabetes. The Uric acid levels are significantly increased in ISPs when compared to control subjects($p<0.003$) and the increase is more in ISPs with diabetes (Table 1). The correlation between MDA and antioxidants GSH, uric acid and ceruloplasmin is given in Table2.

DISCUSSION

The present study shows significant increase in lipid peroxides in Ischemic stroke Patients (ISPs), compared to control subjects. ISPs without diabetes showed highest levels of lipid peroxides when compared to ISPs with diabetes. Increased lipid peroxides and oxidative stress has been implicated as a potential contributor to the pathogenesis of active CNS injury (14). After brain injury by ischemic or hemorrhagic stroke, the production of reactive oxygen species (ROS) may increase leading to tissue damage. These radicals can cause damage to cardinal cellular components such an lipids, proteins and nucleic acids leading to subsequent cell death by necrosis or apoptosis.

Some of the pathological processes in acute CNS injury involve the generation of oxygen free radicals either as a cause or a result of disease progression (15). Free radicals are generated by the constant use of oxygen in the mitochondria . Some enzymes expressed in the brain including monoamine oxidase produce H_2O_2 as normal by products of their activity(16). The activity of other neuronal enzymes yields oxidants . Auto-oxidation of endogenous substance like ascorbic acid and catecholamine, may yield high levels of H_2O_2 (17). These free radicals and related ROS mediate much of the damage that occurs after brain ischemia. Demirkaya et al (18) showed that patients with acute ischemic stroke had significantly higher levels of MDA in their red blood cells after stroke onset, compared with controls. In line with these authors we have also found in our study that MDA levels are highly increased in ISPs with and without diabetes.

Table 1 : Comparison of MDA, GSH, Uric acid and Ceruloplasmin in ISP patients with and without diabetes and Control subjects

Parameters		Control Mean± SD	ISP without diabetes Mean ± SD	ISP with diabetes Mean ± SD
1.	MDA (nmoles/dL)	384.118± 90.353 (211.10 – 502.39)	854.958 ± 176.859 (490.59 -1076.92) P < 0.001	678.866±273.125 (48.63 – 1232) P < 0.001
2.	GSH (mg/dl)	54.928± 7.362 (39.60-65.50)	22.676 ± 8.548 (9.16 - 36.68) P < 0.001	17.37±9.769 (4.08- 36.86) P < 0.001
3.	Ceruloplasmin (mg / dl)	37.798± 5.853 (27.20 -46.90)	44.704 ± 13.497 (20.10 - 58.28) P < 0.016	35.491 ±13.508 (15.71 – 66.13) P < 0.317
4.	Uric acid (mg / dl)	5.2165 ± 1.505 (2.15 – 7.53)	6.909 ±1.441 (5.00 - 10.20) P < 0.003	8.709 ± 1.529 (6.50- 11.40) P < 0.003

SD= Standard Deviation; p-value calculated by Mann Whitney test; The figures in the parentheses indicates the range.

Diabetics are known to have an increased susceptibility to coronary, femoral and cerebral artery atherosclerosis and also increased risk of stroke. Although diabetes is a strong risk factor for stroke, it is still unclear whether the extent of lipid peroxidation is same or different in diabetic or non diabetic stroke patients. Our study indicates that lipid peroxides are increased in both the groups and the increase is more in ISPs without diabetes.

Antioxidants are exogenous or endogenous compounds acting in several ways, scavenging reactive oxygen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation. Recently increased oxidative stress and impaired anti oxidant defense have been suggested as a contributory factor for initiation and progression of complications in schizophrenia, diabetes, malaria and coronary artery diseases (6,19,20,21). To study the antioxidant potential of ischemic stroke patients we have included Glutathione (GSH), Uric acid and Ceruloplasmin in our study.

Our results indicate that GSH levels are decreased significantly in ISPs and maximum decline is observed in ISPs with diabetes. GSH depletion enhances cerebral ischemic injury. Shivakumar et al (22) and Akila et al (21) have shown that GSH levels have decreased in brain regions during reperfusion for 1 h after moderate or severe ischemia for 0-5 hours. The GSH was shown to reduce lethality, increase brain water levels and decrease MDA levels in cerebral ischemic rats when given immediately after ischemia suggesting that its anti ischemic effects are due, in part to inhibition of lipid peroxidative responses (23,24). In our study we have found decreased GSH levels in ISPs with and without diabetes which indicates that the antioxidant capacity is decreased in these patients. There is a negative correlation between MDA and GSH in ISPs with diabetes. Therefore treatment with anti oxidant might be beneficial to reduce MDA in ischemic stroke patients.

We have also found in our study that ceruloplasmin levels are increased in ISPs without diabetes when compared to control

Table : 2 CORRELATIONS

GROUP			GSH (mg/dl)	Uric acid (mg/dl)	Ceruloplasmin (mg/dl)
Controls	MDA	r	-0.221	0.115	- 0.047
		p	0.349	0.628	0.844
		n	20	20	20
ISP without diabetes	MDA	r	0.070	-0.031	0.187
		p	0.805	0.914	0.505
		n	15	15	15
ISP with diabetes	MDA	r	-0.151	-0.394	-0.205
		p	0.509	0.146	0.463
		n	15	15	15

subjects, where as its levels are decreased in ISPs with diabetes. Cerulopasmin is the primary antioxidant and barrier against free radicals in the blood stream. Ceruloplasmin ferroxidase activity is of greatest importance in conversion of Fe^{++} to Fe^{+++} and may reduce oxidation by inhibition of the Fenton reaction. Cenuloplasmin inhibits the oxidation of lipids, poly unsaturated fatty acids and phospholipids. Ceruloplasmin antioxidant activity also blocks protein and DNA damage and removes reactive intermediate hydrogen peroxide. Increase in the levels of ceruloplasmin in ischemic stroke patients without diabetes may be due to an adaptive mechanism to increased oxidative stress. A decrease in the ceruloplasmin levels as observed in ISPs with diabetes indicates that antioxidant defense is impaired in these patients(25).

Our study also indicates that uric acid levels are significantly increased in ISPs with and without diabetes and the increase is more in ISPs with diabetes. Uric acid, which is the end product of purine metabolism has been regarded as potent endogenous water soluble antioxidant and radical scavenger in humans (26). Uric acid plays an important role in acute ischemic stroke, as a consequence of its antioxidant properties. It is particularly effective in quenching hydroxyl superoxide and peroxynitrite radicals and may serve a protective physiological role by preventing lipid peroxidation (26).

An association between raised serum uric acid concentration and increased cardio-vascular risk has been recognized. Raised serum uric acid concentrations are associated with increased risk of stroke in high risk patient groups like hypertension, cardiovascular diseases and diabetes mellitus(27) . It is unclear whether high uric acid concentration promotes or protects against the development of ischemic stroke or simply act as a passive marker of increased risk. In our studies we have found increase in uric acid concentration in ISPs and increased uric acid concentration during acute oxidative stress and ischemia, might be a compensatory mechanism that confer protection against increased free radical activity.

From these studies we can conclude that antioxidant defense is impaired in ischemic stroke patients as a consequence of increased oxidative stress.

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