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Original Article

Role of Oxidative Stress in Pathophysiology of Transient Ischemic Attack and Stroke

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ABSTRACT

Background Oxidative stress, characterized by increased generation of oxygen free radicals results in generation of lipid peroxides like malondialdehyde (MDA). Enzymatic and nonenzymatic antioxidant enzymes like superoxide dismutase (SOD) and Vitamin E are part of intracellular protection mechanisms to overcome oxidative stress. The present study evaluated lipid profile, oxidative stress and antioxidants in Transient ischemic attack (TIA) and ischemic stroke patients. Method: The study was carried out on 50 diagnosed cases of acute ischemic stroke, 10 cases of TIA and 60 healthy controls. Fasting blood samples were collected and assessed for serum lipid profile (serum cholesterol, triglycerides, serum HDL and serum LDL cholesterol) and markers of lipid peroxidation (MDA) and antioxidants (Erythrocyte SOD and serum Vitamin E). Statistical analysis was performed using Spss 13. P-values less than 0.05 were considered significant. Result: In stroke patients as well as TIA patients, a significant increase was observed in plasma sugar, total cholesterol, triglycerides , LDL cholesterol and MDA whereas a significant decrease was observed in serum HDL, erythrocyte SOD and Vitamin E levels as compared to control group. The patients with TIA demonstrated more deranged lipid profile as compared to stroke patients whereas stroke patients had higher oxidative stress than TIA patients as evidenced by higher levels of MDA and lower levels of SOD and Vitamin E in stroke patients as compared to TIA patients. Conclusion: Oxidative stress is a potential contributor to acute ischemic stroke and TIA besides deranged lipid profile. The oxidant-antioxidant imbalance may contribute to the severity of stroke.

1. Introduction

Stroke is one of the most common disabling neurological disorders and is one of the leading causes of death in most countries together with coronary artery disease [1]. Stroke is a major cause of morbidity and mortality in an aging population. In the elderly, ischemic stroke accounts for more than 80% of all stroke cases [2]. India will face enormous socio–economic burden to meet the costs of rehabilitation of "stroke victims" because the population is now surviving through peak years (age 55–65 years) of occurrence of stroke [3]. A transient ischemic attack (TIA) is an episode of temporal and focal cerebral dysfunction of vascular origin, sometimes called a mini-stroke, with stroke symptoms that lasts less than 24 hours before disappearing [4]. While TIAs generally do not cause permanent brain damage, they are a serious warning sign of stroke [5].

While the causes of cellular injury following ischemia are multifactorial, there is now increasing evidence to suggest that reactive oxygen species- superoxide anion, hydrogen peroxide and highly reactive hydroxyl radical may play a key role in its pathogenesis. Oxidative stress resulting from generation of reactive oxygen species is involved in neuronal damage induced by ischemia-reperfusion, and the antioxidant activity of plasma may be an important factor providing protection from neurological damage caused by stroke-associated oxidative stress [6]. Increased free radical formation together with a reduced antioxidant defense causes oxidative stress. This may play a pivotal role in formation of reactive intermediates like superoxide,

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hydroxyl radical, hydrogen peroxide and lipid peroxides – Malondialdehyde (MDA) and hydroperoxides which contribute to the pathogenesis of stroke associated neuronal injury [7,8]. Superoxide radicals formed during oxidative stress may react with various biomolecules resulting in either direct damage or synthesis of potentially harmful products. Increased vascular reactive oxygen species production, especially superoxide anion, contributes significantly in the functional and structural alterations present in hypertension [9].

Superoxide Dismutase (SOD) is reported to be the major enzymatic defence against free radicals and common oxidants [10]. It is the major extracellular scavenger of superoxides, and one of the main regulators of nitric oxide bioactivity in vessel walls [11]. However, superoxide dismutase activity alone may be considered ineffective in balancing oxidative stress, since it leads to synthesis of more reactive hydrogen peroxide. Vitamin E is a lipid soluble peroxyl radical scavenger in human cells. Vitamin E interrupts lipid peroxidation by scavenging peroxyl radical intermediates [12].

Vitamin E may inhibit cell mediated LDL oxidation by reducing cellular production and release of reactive oxygen species. Beneficial effects of vitamin E include inhibition of smooth muscle cell proliferation, preservation of endothelial function, inhibition of monocyte-endothelial adhesion. Though Vitamin E levels have been evaluated in cases of coronary artery disease but the studies in cases of transient ischemic attack and stroke are very few.

The present study evaluated the oxidative stress and antioxidant status in patients of transient ischemic attack, acute ischemic stroke and control subjects with an aim to understand the pathophysiological role of oxidants and antioxidants in stroke.

2. Materials and methods

This study was conducted on 120 subjects and carried out in the Departments of Biochemistry and Neurology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Shahdara, New Delhi. The study subjects included- 50 cases of stroke, 10 cases of transient ischemic attack and 60 age and sex-matched healthy controls. The study was approved by Institutional Ethical Committee and the samples were collected after an informed consent. The acute ischemic stroke was diagnosed on the basis of clinical examination and confirmed by the either initial CT scan of brain or delayed CT scan of brain where first CT scans were normal.

All subjects were examined by a cardiologist and information on medical histories, age, weight, height, BMI, systolic and diastolic pressure, cigarette smoking, habits, recent use of vitamin E and medications were obtained via questionnaire and patients medical records.

Patients with renal, liver, thyroid, gout, diabetes, or malignant disease were excluded from the study. To distinguish infarction from haemorrhage clinically, Siriraj Stroke score was used which provides 95% accuracy [15]. It is calculated as (2.5x level of consciousness) + (2x vomiting) + (2 x headache) + (0.1 x diastolic blood pressure) – (3 x atheroma markers) – 12.

2.1. Blood collection and storage

Blood sample for the estimation of all the parameters was collected from the patients suffering from stroke after an overnight fasting (12-14 hours) whereas in cases of TIA sample was drawn as and when patient came to the hospital. Approximately 10 ml of venous sample was collected from each subject using aseptic precaution. 1 ml sample was collected in sodium Fluoride: oxalate vial for estimation of plasma sugar. Samples for estimation of SOD were collected in EDTA vial where as all other investigations were carried out on sera collected in plain vials. Samples were allowed to clot and serum was separated by centrifugation at 2500 rpm for 15 minutes. Serum lipid profile and baseline liver function tests and renal function tests were carried out immediately. Remaining sera was stored at -20°C until analysed for MDA and Vitamin E. Hemolysed or icteric samples were rejected.

2.2. Estimation of serum MDA

MDA was measured as Thiobarbituric acid (TBA) activity by using the colorimetric method recommended by Buege and Aust cited by Valenzuela [13].

2.3. Estimation of Erythrocyte Superoxide Dismutase (SOD)

The activity of SOD in erythrocytes was determined by the method described by Marklund and Marklund as modified by Nandi and Chatterjee [14]. The procedure included washing erythrocytes twice with normal saline and preparation of hemolysate using 1.5 volumes of cold distilled water. Hemoglobin concentration was measured by Cynmethemoglobin method using Drabkin's reagent and was adjusted to 10 gm per 100ml. Enzyme activity was measured by inhibition of pyrogallol auto-oxidation. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3 ml of assay. Results have been expressed in unit/gm Hemoglobin for erythrocyte hemolysate.

2.4. Estimation of serum Vitamin E (Serum tocopherol)

Serum tocopherol was determined by the method of Baker and Frank [15]. Serum tocopherol can be determined by the reduction of ferric to ferrous ions which then form a red complex with α, α-dipyridyl. Tocopherol and carotenes are first extracted into xylene and the absorbance is read at 460 nm to measure the carotenes. A correction for carotenes is made after adding ferric chloride and reading is made at 520nm.

2.4. Estimation of serum Vitamin E (Serum tocopherol)

Serum total cholesterol, triglycerides and serum HDL were estimated by enzymatic method on Beckmann CX4 autoanalyser using standard kits and reagents from Randox (UK). Serum LDL was calculated using Friedewald and Fredrickson's formula [16].

2.6. Statistical analysis

All statistical analysis was performed using SPSS 13. Results are expressed as Mean ± Standard error of mean. Data were tested for normal distribution with the Kolmogorov-Smirnov test. Differences were compared using student -test and ANOVA for parameters which showed a normal distribution and Mann-Whitney test for parameters which did not show normal

distribution. Relationships between parameters were determined by Pearson's correlation coefficient. The values of ($p<0.05$) were taken as significant.

3. Results

The study population included 50 patients of acute ischemic stroke in the age group of 40 to 89 years whereas the presenting age group of patients with transient ischemic attack was 50- 89 years. In both the groups, maximum incidence was observed in the age group 60-69 years. Among the stroke patients, 54% were men and 46% were women. In the transient ischemic attack group, 60% patients were men and 40% women. The control group also included 56% men and 44% women in the age group 45- 89 years.

In stroke patients as well as transient ischemic attack patients, a significant increase was observed in plasma sugar, total cholesterol, triglycerides , LDL cholesterol and MDA whereas a significant decrease was observed in serum HDL, erythrocyte SOD and Vitamin E levels as compared to control group as shown in Table 1. In this study, the patients with transient ischemic attack demonstrated more deranged lipid profile as compared to stroke patients. On the other hand, stroke patients demonstrated the presence of higher oxidative stress than TIA patients as evidenced by higher levels of MDA and lower levels of SOD and Vitamin E in stroke patients as compared to TIA patients.

Stroke patients were further divided into two groups – thromboembolic stroke (n=42) and haemorrhagic stroke (n=8) based on the Siriraj stroke score. A non-significant difference was observed between markers of oxidative stress between the two groups as shown in Table 2. However, a significant difference was observed between in serum lipid profile between the two groups. The thromboembolic stroke patients demonstrated significantly deranged levels of total cholesterol, triglycerides and serum LDL as compared to haemorrhagic stroke patients.

Table 1. Comparison between plasma sugar, lipid profile and markers of oxidative stress between stroke patients, TIA patients and control group

	Stroke N=50	TIA N=10	Control N=60
Sugar(mg/dl)	114.80±5.20*	124.9±18.17*	94.96±1.35
T Chol(mg/dl)	243.12± 9.55*	255.90±9.77**	195.16±2.89
TG(mg/dl)	213.90±18.74*	254.2±19.38*	147.83±2.21
HDL(mg/dl)	25.36±0.84**	21.20±0.85**	34.21±1.41
LDL(mg/dl)	166.68±9.02*	175.20±10.55*	121.76±3.47
MDA(nmol/L)	4.2±0.26**	3.76±0.38**	2.69±0.39
SOD (U/gHb)	1405.86±51.14**	1517.60±100.98*	2130.16±54.62
Vit E(mg/L)	6.80±0.18*	6.76±0.18*	7.35±0.12

All comparisons are between the study group and the control group * p< 0.05 (significant) , ** p< 0.01 (highly significant)

Table 2. Comparison between lipid profile and markers of oxidative stress between thromboembolic and haemorrhagic stroke patients, TIA patients and control group

Table 5. Trichomoniasis against occupation in the study

	Thromboembolic stroke N=42	Haemorrhagic stroke N=8
MDA(nmol/L)	4.14±0.26	4.47±0.91
SOD (U/gHb)	1426.07±54.55	1299.75±144.92
Vit E(mg/L)	6.82±0.16	6.67±0.45
T Chol(mg/dl)	252.97±10.56*	191.37±10.14
TG(mg/dl)	228.78±20.82*	135.75±31.46
HDL(mg/dl)	24.36±0.63	28.30±1.51
LDL(mg/dl)	175.90±9.84*	118.25±13.61

All comparisons are between the study group and the control group * p< 0.05 (significant) , ** p< 0.01 (highly significant)

A significant positive correlation was observed between serum MDA levels and serum total cholesterol (r= 0.401, p=0.043) and LDL (r=0.432, p=0.032). However, no significant correlation was observed between anti-oxidant status and lipid profile. A significant negative correlation was observed between serum MDA and serum Vitamin E levels in both transient ischemic attack (r= -0.492, p= 0.031) and stroke patients (r=-0.583, p=0.012). A significant negative correlation was observed between serum MDA and erythrocyte SOD levels in the TIA patients (r=-0.452, p=0.044) whereas a non-significant negative correlation was observed in stroke patients (r=-0.294, p=0.208).

4.Discussion

The present study evaluated the role of dyslipidemia and oxidative stress in patients of transient ischemic attack and stroke. The role of hyperlipidemia in CAD is well established,
whereas its association with cerebrovascular disorders is still being evaluated. In the present study, serum cholesterol in stroke cases was 243.12± 9.55 mg/dl. In TIA cases, the value for serum cholesterol was 255.90±9.77 mg/dl. In both these groups, the values were significantly higher than in the control group (195.16±2.89 mg/dl). However, in the haemorrhagic stroke patients the values were comparable to that of the control group.
Similarly LDL Cholesterol and serum triglycerides were also significantly higher in the stroke patients and TIA patients, but in the haemorrhagic stroke patients, these values were in the normal range and comparable to the control group. A similar, statistically positive correlation between serum total cholesterol, triglycerides and LDL cholesterol was reported in a recent study on non-diabetic stroke patients [17]. No direct relationship, long or short term between ischemic stroke and total or LDL cholesterol has been reported in the Framingham study and no protective effect of HDL cholesterol was seen [18]. A report from the atherosclerosis risk in communities (ARIC) study found only weak and inconsistent association between ischemic stroke and each of the five lipid factors in 305 subjects experiencing ischemic stroke after 10 years of prospective investigation [19].

However, the serum lipid levels have been directly related to extracranial carotid artery wall thickness [20,21]. The derangements in lipid profile may promote the development of atheroma in the carotid artery wall and thickening of intima media. A highly significant difference was observed in serum MDA levels between stroke and TIA cases compared to the control group ($p<0.01$), suggesting an increase in lipid peroxides during stroke and TIA. However, serum MDA levels were not significantly different in thromboembolic (4.14±0.26 nmol/L) and haemorrhagic stroke patients (4.47±0.91 nmol/L) ($p=0.12$). The results of the present study are in consistence with another study carried out in Indian population by Beg etal [22] and Huang etal [23] where a significant difference in serum MDA levels was detected between stroke cases and controls but no significant difference was reported between hemorrhagic and thrombotic stroke. However, Santos etal [24] discovered higher MDA-like material in patients of thrombotic stroke as compared to haemorrhagic stroke. The increase in lipid peroxides could result from increased oxidation of blood and/or neural lipids as evidenced by a significant positive correlation between serum MDA and serum total cholesterol as well serum LDL cholesterol. Brain, being rich in lipids could also be predisposed to lipid peroxidation [25]. A significant negative correlation was observed between serum MDA and Vitamin E levels ($p=0.031$). This negative correlation can be explained by the fact that Vitamin E is a peroxyl radical scavenger and thus a decrease in Vitamin E levels may predispose to generation of more lipid peroxidation products. A significantly decreased level of serum Vitamin E and SOD were observed in stroke and TIA cases as compared to controls in this study. The decrease in stroke cases was highly significant compared to TIA where the decrease was significant but lesser than in stroke. The findings of this study are similar to a few studies where decreased SOD levels have been reported in stroke cases [26,27] but in contrast to another study where no increase was reported [28]. In most of the reported studies, mainly stroke patients have been studied but the present study provides a comparison of oxidative stress and anti-oxidant status in TIA and stroke cases. The decrease in SOD levels may result from early depletion of antioxidants as a consequence of an excessive production of oxygen free radicals after the ischemic insult, the depletion being more in large-sized infarcts [26]. Erythrocyte SOD levels had a significant negative correlation with serum MDA in the TIA patients ($r=-0.452$, $p=0.044$) whereas a nonsignificant negative correlation was observed in stroke patients ($r=-0.294$, $p=0.208$). The decreased SOD levels observed in stroke patients can lead to formation of more toxic hydrogen peroxide thus leading to generation of lipid peroxides and greater damage due to oxidative stress. Some studies have shown reduced serum vitamin E levels in stroke patients [27] and this may be due to high lesion volume resulting in production of more number of free radicals from a large ischemic injury. In the present study also, serum vitamin E levels were significantly decreased in ischemic stroke cases as well as TIA cases. No significant difference was observed in serum Vitamin E levels between the two study groups.

The decrease in serum Vitamin E and erythrocyte SOD levels was more in haemorrhagic stroke as compared to thrombo-embolic stroke but the difference in the levels between the two groups was insignificant. Another study carried out in Turkish population also demonstrated a decrease in plasma SOD and total superoxide scavenger activities in acute hemorrhagic stroke [29].

5. Conclusion

Thus the present study indicates that severe depletion in antioxidant system is unable to combat oxidative stress. This antioxidant system could be an important protective system against oxidative damage but tends to be severely impaired in ischemic cerebro-vascular conditions. The findings of the present study indicate that the existence of an abnormal balance between the oxidative and protective mechanisms in patients can be a causative factor for acute cerebral ischemia. This imbalance may be more pronounced in TIA as compared to cases of acute ischemic stroke.

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