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Research Article

EVALUATION OF SERUM ANGIOTENSIN CONVERTING ENZYME, OXIDATIVE STRESS, AND ANTIOXIDANT LEVELS IN CEREBROVASCULAR DISORDERS AND HEALTHY CONTROL − A COMPARATIVE STUDY

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ABSTRACT

Objective: Several studies indicate the existence of an abnormal balance between the oxidative and protective mechanisms in patients can be a causative factor for cerebrovascular disease. High plasma levels of angiotensin-converting enzyme (ACE) contribute to the development of vascular diseases. Serum ACE and antioxidant levels were determined in cerebrovascular disorders (CVD) and compared between CVD and healthy person.

Methods: A cross-sectional study was conducted on 50 cases of stroke and 10 cases of transient ischemic attack (TIA) with 60 controls matched for age and sex. Fasting blood samples were estimated for serum angiotensin-converting enzyme, serum lipid profile, MDA, SOD, and serum Vitamin E). ACE was estimated by spectrophotometry. The levels of ACE were correlated with the severity and outcome of the stroke.

Results: The level of serum ACE was significantly higher in stroke (27.50±1.14U/L) and TIA (31.60±2.02 U/L) as compared to controls (22.61±0.79 U/L). Fasting blood sugar, total cholesterol, triglycerides, LDL cholesterol, and MDA were significantly increased whereas a significant decrease was observed in serum HDL, erythrocyte SOD, and Vitamin E levels in cerebrovascular disease patients as compared to the control group. Statistical $p<0.05$ was considered significant.

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. The levels of serum ACE increase significantly in stroke and TIA patients and are associated with increased severity and worse outcome of cerebrovascular diseases.

Keyw3rds: Cerebrovascular disease, Oxidative stress, Antioxidant, Angiotensin-converting enzyme.

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INTRODUCTION

A cerebrovascular accident is a sudden loss of cerebral perfusion or vasculature. Somewhere around 85% of strokes are ischemic, also with the entirety seeming to be hemorrhagic [1]. Oxidative stress, one of the major causes of CVDs, occurs due to a reduction in antioxidant capacity and the production of reactive oxygen species (ROS). Stroke, along with coronary artery disease, is one of the most common disabling neurological disorders and leading causes of death in most countries. Stroke is the fifth most common cause of death in the United States. Stroke represents nearly 800,000 people per year [2]. Numerous studies have shown that the renin angiotensin system (RAS) plays an important role in the mechanism for maintaining blood pressure and vital organ perfusion. RAS inhibitors have potent anti-atherosclerotic effects, which are mediated by their antihypertensive, anti-inflammatory, antiproliferative, and oxidative stress-lowering properties. In fact, RAS inhibitors are first-line treatments for hypertensive target organ damage [3]. Plasma antioxidant activity may be a significant factor in preventing neurological damage caused by stroke-related oxidative stress [4]. An increased free radical formation may be important in the formation of reactive intermediates such as superoxide, hydroxyl radical, hydrogen peroxide, and lipid peroxides - Malondialdehyde (MDA) and hydroperoxides, all of which contribute to the pathogenesis of stroke-associated neuronal injury [5], [6].

Furthermore, in a healthy state, ROS levels are kept at an optimal level by a balance of enzymatic (superoxide dismutase, glutathione, catalase, and peroxidase) and non-enzymatic (Vitamins C and E) antioxidants. Increased oxidant capacity combined with decreased antioxidant capacity creates an unbalanced environment that results in oxidative stress in a pathological state such as metabolic syndrome. However, because it results in the synthesis of more reactive hydrogen peroxide, superoxide dismutase activity alone may be considered ineffective in balancing oxidative stress. In human cells, Vitamin E acts as a lipid-soluble peroxyl radical scavenger and inhibits lipid peroxidation [7]. Despite the fact that vitamin E levels have been studied in cases of transient ischemic attack and stroke, there have been very few studies. Our main objective was to look for possible risk factors for stroke and compare the roles of ACE and antioxidants in patients with cerebrovascular disease, mainly stroke and transient ischemic attack, to healthy controls.

METHODS

The study was carried out at the Department of Biochemistry, University College of Medical Sciences, and Guru Tegh Bahadur Hospital in Shahdra, Delhi. The study included 50 cases of stroke, 10 cases of TIA, and 60 controls, age- and sex-matched. The WHO definition of stroke was used to diagnose acute ischemic stroke, which was confirmed by a CT scan of the head. All patients underwent a detailed history and clinical examination based on a predesigned proforma. The Institutional Ethical Committee approved the study and the samples were collected after informed consent. Patients with kidney, liver, thyroid, gout, diabetes, or cancer were excluded from the study. To distinguish infarction from hemorrhage clinically, the Siriraj stroke score was used which provides 95% accuracy [8]. It is calculated as (2.5 × level of consciousness) + (2 × vomiting) + (2 × headache) + (0.1 × diastolic blood pressure) – (3 × atheroma markers) – 12 [9].

Sample collection and processing

Fasting venous blood samples were collected from stroke patients after an overnight fast (12–14 h) whereas in cases of TIA sample was drawn as and when the patient came to the hospital. The sample was allowed to clot and then centrifuged at 2500 rpm for 10 min, and stored at –20°C until the sample was analyzed. Serum lipid profile and baseline liver function tests and renal function tests were carried out immediately. Serum angiotensin-converting enzyme was estimated by spectrophotometry. Oxidative stress markers, that is, malondialdehyde (MDA), enzymatic and non-enzymatic antioxidant enzymes such as superoxide dismutase (SOD), and Vitamin E parameters were analyzed. Hemolysed or icteric samples were rejected. MDA was measured as thiobarbituric acid (TBA) activity using the colorimetric method [10].

Statistical analysis

All statistical analysis was performed using SPSS 13. Results are expressed as Mean ± Standard error of the mean. Data were tested for normal distribution with the Kolmogorov-Smirnov test. Differences were compared using the student's t-test, and ANOVA for parameters that showed a normal distribution and Mann–Whitney test for parameters that did not show normal distribution. Relationships between parameters were determined by Pearson's correlation coefficient. The values of ($p<0.005$) were considered significant.

RESULT AND DISCUSSION

The study population included 50 patients with acute ischemic stroke in the age group of 40–89 years whereas the presenting age group of patients with the transient ischemic attack was 50–89 years. In both groups, the 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% of patients were men and 40% were women. The control group also included 56% of men and 44% of women in the age group 45–89 years indicated in Table 1. Our study closely correlates with a study done by Gauri *et al*. (19%), [11] Chitrambalam *et al* (20%) [12]. In the above study, males were most commonly affected by a stroke which is supported by the studies conducted in western countries [13-15]. It also correlates with studies done by Aiyar *et al*. [16], Pinhero *et al*. [17], and Eapen *et al*. [18] who found that the incidence of stroke is more common in males than females.

In Table 2, the present study evaluated the role of dyslipidemia and oxidative stress in patients with transient ischemic attack and stroke. The role of hyperlipidemia in CAD is well established, but 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). Similarly, LDL cholesterol and serum triglycerides were also significantly higher in the stroke patients and TIA patients compared to the control group. A similar, statistically significant correlation was reported between serum total cholesterol, triglycerides, and LDL cholesterol recent study done on non-diabetic stroke patients [19]. The level of serum angiotensin-converting enzyme was significantly higher in stroke patients and TIA as compared to controls, though the mean values for both the study groups were found to fall within the reference range. Not many studies have evaluated ACE levels in stroke or TIA patients, but several studies reporting a beneficial effect of the use of ACE inhibitors in the prevention of primary stroke [3,20,21]. Provide indirect evidence of the role of angiotensin-converting enzymes in the pathophysiology of stroke as shown in Table 3.

Derangements in the lipid profile may promote the development of atheroma in the carotid artery wall and intima-media thickening. Serum MDA levels differed significantly between stroke and TIA cases and the control group ($p<0.01$), indicating an increase in lipid peroxides during stroke and TIA. The present study's findings are consistent with those of another study conducted in the Indian population by Beg *et al*. [22] and Huang *et al*. [23], which found a significant difference in serum MDA levels between stroke cases and controls. A significant negative correlation was observed between serum MDA and Vitamin E levels ($p<0.031$). This inverse relationship 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 the generation of more lipid peroxidation products. In this study, stroke and TIA patients had significantly lower levels of serum Vitamin E and SOD than controls. The reduction in stroke cases was highly significant when compared to TIA, where the reduction was significant but less than in stroke, as shown in Table 4. The findings of this study are similar to a few studies that have reported decreased SOD levels in stroke cases [24,25] but differ from another study that found no increase [26]. Most reported studies have focused on stroke patients, but the present study compares oxidative stress and anti-oxidant status in TIA and stroke cases [24].

The present study's findings suggest that an abnormal balance between the oxidative and protective mechanisms in patients can be a causative factor for acute cerebral ischemia. ACE inhibitors provide indirect evidence that ACE plays a role in myocardial infarction. We also discovered that the relationship between various risk factors and

Table 1: Gender distribution among the study group

Group	Men (%)	Women (%)
Stroke	54	46
TIA	60	40
Control	56	44

Table 2: Status of biochemical parameters in stroke and TIA

Parameter	Stroke (Mean±SD)	TIA (Mean±SD)
Serum angiotensin-converting enzyme level	27.50±1.14 U/L	27.50±1.14 U/L
MDA	4.2±0.26**	3.76±0.38**
SOD	1405.86±51.14**	1517.60±100.98*
Vitamin E	6.80±0.18*	6.76±0.18*
FBS	114.80±5.20*	124.9±18.17*
T. cholesterol	243.12±9.55	255.90±9.77**
TG	213.90±18.74*	254.2±19.38*
HDL	25.36±0.84**	21.20±0.85**
LDL	166.68±9.02*	175.20±10.55*

**Highly significant, $p<0.005$

Table 3: Comparison serum level of ACE in stroke, TIA patients with healthy control

Group	Serum angiotensin converting enzyme (U/L) Mean±SEM	p<value
Control	22.61±0.79 U/L	
Stroke	27.50±1.14** U/L	<0.004
TIA	31.60±2.02** U/L	<0.004

**Highly significant, $p<0.005$

Table 4: Comparison between markers of oxidative stress in stroke patients, TIA patients, and control group

Markers	Stroke (Mean±SD)	TIA (Mean±SD)	Control (Mean±SD)
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
Vitamin 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)

the incidence of stroke in relation to age and gender was investigated. The study's limitation was a small number of patients, which can be overcome by enrolling a large number of populations in research studies.

CONCLUSION

As a result, the present study found that severe antioxidant depletion renders the antioxidant system incapable of combating oxidative stress. In ischemic cerebrovascular conditions, the antioxidant system's ability to protect against oxidative damage is severely compromised. Furthermore, ischemic stroke is more common in men. Serum ACE levels are raised in cerebrovascular disorders and may play a role in their pathophysiology. The present study provides clinical evidence that cannot be ignored concerning the involvement of the Renin-Angiotensin System, oxidative stress, and antioxidant status in stroke and TIA. It has the potential to be used as a diagnostic and screening tool to help reduce the burden of stroke.

AUTHORS CONTRIBUTIONS

The manuscript writing had accomplished by Jaspreet Kaur and the data collection and analysis were done by Jaswant Kaur. The research was reviewed and edited by Mittal and statistical analysis was done by Gupta. The manuscript was finalized and submitted for publication by Jaswant Kaur.

CONFLICTS OF INTEREST

The authors affirm no conflicts of interest.

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