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RESEARCH ARTICLE

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ANGIOTENSIN CONVERTING ENZYME LEVELS IN ISCHEMIC CEREBROVASCULAR DISORDERS: A CASE-CONTROL PILOT STUDY IN NORTH INDIAN POPULATION

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ABSTRACT

Background: Experimental studies in lower animals indicate that high plasma levels of angiotensin converting enzyme (ACE) contribute to development of vascular diseases. The level of serum ACE was determined in cerebrovascular disorders [stroke and transient ischemic attack (TIA)].

Method: The present cross-sectional study was carried out on 50 cases of stroke and 10 cases of TIA with 60 controls matched for age and sex. Serum angiotensin converting enzyme was estimated by spectrophotometric measurement using the synthetic tripeptide substrate (N-(3) (2 - furyl)adroyloyl) –L- phenylalanylglycyl glycine(FAPGG). The levels of ACE were correlated with severity and outcome of stroke.

Result

The level of serum ACE were 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). Higher levels of ACE showed positive correlation with severity of disease and poorer outcome.

Conclusion

Levels of serum ACE increase significantly in stroke and TIA patients and is associated with increased severity and worse outcome of cerebrovascular diseases.

KEY WORDS

Angiotensin converting enzyme (ACE), Stroke, Transient Ischemic attack, lacunar infarct, outcome.

INTRODUCTION

Stroke is defined by the National Institute of Neurological Disorders and Stroke (NINDS) as a sudden loss of brain function resulting from an interference of blood supply to the brain [1]. Transient ischemic attack, also considered as pre-stroke, is a brief episode of neurological dysfunction caused by focal brain or retinal ischemia with clinical symptoms typically lasting less than 1 hour and without evidence of acute infarction on brain scanning [2].

Hypertension has been identified as one of the risk factors for stroke, accounting for 25-49% of all cases [3]. Hypertension predisposes to the cardiac conditions promoting haemorrhage, cerebral embolism and to a lesser extent to subarachnoid haemorrhage from aneurysm. The renin angiotensin system (RAS) has an important role in the mechanism for maintenance of blood pressure and perfusion of vital organs. Recent studies have shown that RAS blocking agents have potent antiatherosclerotic effects, which are mediated by their antihypertensive, anti-inflammatory, antiproliferative, and oxidative stress lowering properties. Inhibitors of RAS are in fact the first-line treatments for hypertensive target organ damage [4].

In a meta-analysis of seven placebo-controlled trials of ACE inhibitors in patients with coronary heart disease and/or diabetes mellitus, the overall risk of primary stroke was significantly reduced [5]. Results of the Heart Outcomes Prevention Evaluation (HOPE) trial have shown a substantial reduction in risk of stroke and transient ischemic attack with ramipril despite an apparently small reduction in blood pressure, suggesting that the benefit of ACE inhibitors may be related to their effects on the renin-angiotensin-aldosterone system more than on blood pressure reduction [6].

The various fore-mentioned studies involving ACE inhibitors provide an indirect evidence of the role of angiotensin converting enzymes and their end products (angiotensin II) in vascular lesions. However, very few studies have evaluated serum angiotensin converting enzyme levels in stroke patients. Hence the present study was undertaken to evaluate serum ACE levels using an easy spectrophotometric method, in patients of cerebrovascular disorders mainly stroke and transient ischemic attack.

Method

The study was conducted in the Department of Biochemistry, University College of Medical Sciences and Guru Tegh Bahadur Hospital,Shahdra,Delhi. The study was carried out in 50 cases of stroke and 10 cases of TIA and 60 controls matched for age and sex.

Acute ischemic stroke was diagnosed by WHO definition of stroke and confirmed by CT scan of head. Based on the CT scan the patients of stroke were categorized into either lacunar or cortical infarct. A detailed history and clinical examination was done in all patients based on a predesigned proforma. The outcome of stroke patients was assessed by presence of either of these on follow up- full recovery, partial recovery, death.

To distinguish infarction from haemorrhage clinically, Siriraj stroke score was used which provides 95% accuracy [7]. It was calculated using the formula

(2.5 x level of consciousness)+(2xVomiting) +(2x headache)+(0.1xdiastolic blood pressure)(3 x atheroma markers) – 12 [8].

The study was approved by Institutional Ethical Committee and the samples were collected after an informed consent.

Sample Collection and processing

Fasting venous blood samples were collected from stroke patients after an overnight fast (12-14 hours) 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 10min.Serum was stored at -20˚C until the batch was analyzed.

Estimation of serum Angiotensin Converting Enzyme

Serum angiotensin converting enzyme was estimated by spectrophotometric measurement using the synthetic tripeptide substrate (N-(3) (2 - furyl) adroyloyl) –L- phenylalanylglycyl glycine as a substrate (FAPGG) [9]. ACE hydrolyzes FAPGG to furylacryloyl- α-phenylalanine (FAP) and glycylglycine and this hydrolysis is associated with a decrease in absorbance at 340nm. The ACE activity in the sample is determined by comparing the sample reaction rate to that obtained with ACE calibrator.

Statistical Analysis

All statistical analysis was performed using SPSS 13. Results are expressed as Mean ± Standard error of mean. Difference in ACE values between different groups was assessed using ANOVA Relationships between ACE levels and outcome of the disease was determined by Pearson's correlation coefficient. The values of ($p<0.05$) were taken as significant.

RESULT

The subjects selected for the studies were age and sex matched for all the three groups. The gender based ratio (male: female) was 1: 1.17 (stroke patients), 1: 1.5 (TIA patients and 1: 1.22 (in control group).

ACE levels were found to be significantly higher in stroke patients and TIA patients when compared to control group (Table1). Based on the Siriraj Score, 8 patients were identified as hemorrhagic stroke and rest 42 patients were diagnosed as thromboembolic stroke. The Angiotensin Converting Enzyme (ACE) levels were significantly higher in thromboembolic stroke as compared to hemorrhagic stroke (Table 2).

Table 1

Comparison of serum Angiotensin converting enzyme (ACE) levels in stroke, TIA and control group.

Group	S.Angiotensin converting enzyme (U/L) Mean ± SEM	P-Value
Control (n=60)	22.61± 0.79 U/L	
Stroke (n=50)	27.50 ± 1.14U/L	P= 0.004
TIA (n=10)	31.60 ±2.02 U/L	P< 0.001

Table 2

Comparison Serum Angiotensin converting enzyme levels in thromboembolic and hemorrhagic stroke

	Serum Angiotensin converting enzyme level (U/L)	P-value
Thrombo-embolic Stroke(42)	28.50 ± 1.25	P = 0.021
Hemorrhagic stroke (8)	22.25 ± 2.21	

ACE values showed a significant positive correlation with severity of disease (cortical/ Lacunar infarction) and outcome of stroke (full recovery/ partial recovery/ death). (Table3).

Table 3
Correlation of ACE values with severity of stroke and outcome

ACE levels (U/L)	Number of patients	Severity			Disease Outcome			
		Lacunar infarct	Cortical infarct	p-value	Full recovery	Partial recovery	Death	Significance
<20	19	12	7		11	8	0	R=0.412
>20	31	12	19	0.028	8	16	7	P= 0.035

DISCUSSION

The present study evaluated and compared serum angiotensin converting enzyme levels in stroke patients, transient ischemic attack patients and healthy controls.

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 use of ACE inhibitors in prevention of primary stroke [3, 5, 6] provide an indirect evidence of the role of angiotensin converting enzyme in pathophysiology of stroke. The levels of serum ACE were apparently higher in TIA patients as compared to stroke but the difference in the two study groups was non-significant. The apparent difference could result from the lesser number of patients in TIA group as compared to stroke.

Hemorrhagic strokes appear to be directly related to the level of blood pressure elevation. Whereas ischemic stroke is largely accounted for by atherosclerotic lesions of the extracranial and/or intracranial cerebral arteries and by arteriosclerotic changes in small cerebral arteries, which are the hallmark of uncontrolled hypertension, i.e., accelerated atherosclerosis [10]. The detection of significantly higher levels of ACE in thromboembolic stroke patients compared to hemorrhagic stroke patients indicate the involvement of ACE in various pathophysiological mechanisms leading to development of atherosclerotic lesions in cerebral arteries in such patients. Several studies on ACE inhibitors provide an indirect evidence of role of ACE in infarction. ACE inhibitors have been found to decrease collagen and elastin accumulation and the cross-sectional wall area of the media in arteries of growing Wistar rats independent of their effects on blood pressure [11]. ACE inhibitors have been shown to reduce atherogenesis in rabbits [12] as well as in cholesterol-fed monkeys [13] and the prevalence of stroke and kidney dysfunction in spontaneously hypertensive rats [14].

In the present study, increased levels of ACE correlated positively with increased severity (presence of cortical stroke) and worse outcome (death and partial recovery) in stroke patients. When serum ACE levels were less than 20 U/L at initial presentation of the patient, no death was reported on follow-up and only 7 out of 19 patients had partial recovery. Whereas, when serum ACE levels were more than 20U/L, 16 out of 31 patients had partial recovery and 7 patients died during hospitalization. These findings reinforce the protective role of ACE inhibitors and angiotensin II receptor blockers (ARB) in stroke prevention as reported in several recent studies.

CONCLUSION

Serum ACE levels are increased in cerebrovascular disorders and may play a major role in pathophysiology of these disorders. The present study provides a clinical evidence of the role of Renin Angiotensin System in stroke and TIA.

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