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Original Article

Biochemical Study of Antioxidant Profile in Acute Ischemic Stroke

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Abstract

BACKGROUND : The present study was designed to measure changes in markers of antioxidant capacity (measured individually and total) following acute ischemic stroke.

METHODS : The study included 135 subjects. 62 were controls and 73 were ischemic stroke patients diagnosed clinically and by CT scan of the brain. The cases were divided into two groups, The ischemic stroke patients with large vessel / cortical, subcortical infarcts (Group. I) and small vessel / lacunar infarcts (Group. II) based on CT scan of the brain. Serum vitamin E, vitamin C, superoxide dismutase, uric acid and total antioxidant capacity were estimated in all the subjects.

RESULTS : Group I and Group II ischemic stroke cases had significantly lower levels of vitamin E, vitamin C and superoxide dismutase and significantly higher levels of uric acid compared to controls. The group I ischemic stroke cases had significantly lower levels of vitamin E, vitamin C, and superoxide dismutase and significantly higher levels of uric acid than group II ischemic stroke cases. Total antioxidant capacity strongly correlated with serum uric acid in cases

CONCLUSION: The present study suggests that estimation of vitamin E, vitamin C, SOD, uric acid and total antioxidant capacity may be used as an indirect evidence of oxidative stress induced neuronal damage in acute ischemic stroke which may be useful for monitoring and optimizing antioxidant therapy.

KEY WORDS: Stroke, oxidative stress, SOD, vitamin C, vitamin E. Total antioxidant capacity.

Several studies provide evidence of an association between ischemic stroke and oxidative stress. Increased free radical formation together with a reduced antioxidant defense causes oxidative stress, that may play an important role in the pathogenesis of stroke associated neuronal injury. Several studies demonstrate increased oxidative damage to neuronal cells during cerebral ischemia and reperfusion. Antioxidant activity is known to reflect the altered redox balance of affected fluids, tissues or organs in acute ischemic stroke patients. Therefore antioxidant concentrations or measures of their activity have been used to estimate the amount of oxidative stress [1]. No single component of serum antioxidant complex could fully reflect the protective efficiency of blood, probably because of interactions that occur *in vivo* among different antioxidant compounds. Total antioxidant capacity considers the cumulative effect of all antioxidants present in blood and body fluids [2]. The aim of this study was therefore to measure changes in markers of antioxidant capacity (measured individually and total) following acute ischemic stroke.

Materials and methods:

This study was conducted at Bapuji Hospital and Chigateri General Hospital, Davangere (Both Hospitals attached to J.J.M. Medical College, Davangere, karnataka), by including 62 healthy controls (of which 34 were men and 28 were women aged between 36 and 73 years) and 73 cases clinically diagnosed as acute ischemic stroke patients of less than 48 hrs duration after the onset of symptoms) and confirmed by computerized tomography of the brain (of which 41 were men and 32 were women aged between 36 and 73 years). The cases were divided into two groups, The ischemic stroke patients with large vessel / cortical, subcortical infarcts (Group. I) and small vessel/lacunar infarcts (Group. II) based on CT scan of the brain. The stroke patients due to cerebral hemorrhage, malignancy, sepsis, severe medical or psychiatric illness, language disorders, swallowing difficulties, cognitive impairment, gout, renal failure and patients who were taking antioxidant vitamins were excluded from the study. The study was conducted after informed consent was obtained from them and the study has been approved by the ethical committee of the institution.

Under aseptic precautions about 6 ml of a non-fasting venous blood sample was collected from cases within 24 h following stroke onset and from healthy controls. Blood was collected in appropriate tubes and centrifuged at 3000 g for 15 min to separate plasma from red blood cells. The supernatant was stored at 4^0 C until analysis was carried out.

Serum vitamin E was estimated by Baker and Frank method [3], Vitamin C by 2, 4 – DNPH method [4], SOD by Marklund and Marklund method [5], and uric acid by Henry Caraway method [6] and total antioxidant capacity (TAC) by FRAP assay method [2] in both the controls and cases. All the chemicals used were of highest analytical grade available in India.

TABLE I. Comparison of Serum Vitamin E, Vitamin C, SOD, Uric Acid and Total Antioxidant Capacity (Mean ± SD)					
	Vit. E mg/L	Vit. C mg/dl	SOD units/ml	Uric acid mg/dl	TAC (µmol/l)
Controls (n = 62)	11.04 ± 0.97	1.16 ± 0.13	9.01 ± 1.03	4.66 ± 0.47	1079.7± 197.9
Cases (n = 73)	7.22 ± 0.81	0.52 ± 0.16	4.35 ± 0.70	6.56 ± 0.73	1043.4± 140.7
Comparison	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$

TABLE II. Comparison of Serum Vitamin E, Vitamin C, SOD, Uric Acid and Total Antioxidant Capacity (Mean ± SD) between Group I and Group II Cases.					
	Vit. E mg/L	Vit. C mg/dl	SOD units/ml	Uric acid mg/dl	TAC (µmol/l)
Cases Group. I (n = 56)	6.93 ± 0.67	0.45 ± 0.08	4.14 ± 0.61	6.78 ± 0.62	1048.9 ± 140.3
Cases Group. II (n = 15)	8.24 ± 0.17	0.80 ± 0.10	5.12 ± 0.49	5.73 ± 0.55	1038.1 ± 142.9
Comparison (one way ANOVA and student 't' test)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$

Statistical analysis :

Statistical analysis was performed with one way ANOVA test, student "t" test and Pearson's correlation coefficient using SPSS version 16.0. A value of $p < 0.05$ was taken to indicate statistical significance.

Results:

It was observed that the serum levels of Vitamin E, Vitamin C, TAC and SOD were significantly lower in ischemic stroke cases than those of controls and serum uric acid levels were significantly higher in ischemic stroke cases [table I]. Further it was observed that the group I ischemic stroke cases had significantly lower levels of serum Vitamin E, Vitamin C and SOD than group II ischemic stroke cases and significantly higher serum levels of uric acid in group I cases than group II ischemic stroke cases [table II]. Significant negative correlations were observed between vitamin C, vitamin E, SOD and TAC and significant positive correlation was observed between uric acid and TAC among cases [table III].

TABLE III. Correlations of Vitamin E, Vitamin C, SOD, Uric Acid with Total Antioxidant Capacity in cases.				
	Vitamin C and TAC	Vitamin E and TAC	SOD and TAC	Uric acid and TAC
R value	-0.24	-0.1	-0.19	0.16
p value	< 0.05	< 0.05	< 0.05	< 0.05

Discussion:

In this study, there were reduced concentrations of vitamin E, vitamin C, SOD and TAC and increased concentrations of uric acid in stroke patients compared with controls. FRAP assay is presented as a novel method of assessing total antioxidant capacity [2] which is believed to be a useful measure of the ability of antioxidant present in the fluids to protect against oxidative damage to membranes and other cellular components..

Vitamin E, a potent chain breaking lipid soluble antioxidant, reacts with lipid peroxyl radicals eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage. Some studies have shown reduced serum vitamin E levels in stroke patients and this may be due to high lesion volume resulting in production of more number of free radicals from a large ischemic injury. It is also shown that reduced vitamin E levels resulted in poor clinical outcome in stroke patients [7,8]. In the present study serum vitamin E levels were significantly decreased in ischemic stroke cases (significantly decreased in large vessel infarcts than in small vessel infarcts) when compared to controls.

Vitamin C represents the major water-soluble antioxidant in the human body. Many studies show that reduced vitamin C levels are associated with increased risk of both ischemic and hemorrhagic strokes [9]. In our present study the serum vitamin C levels were decreased significantly in ischemic stroke cases (decreased significantly in large vessel infarcts than in small vessel infarcts) compared to controls. It may be due to the exhaustion of this antioxidant in the neutralization of free radicals which are formed in excess during ischemia and reperfusion [10,11].

SOD is an endogenous antioxidant that catalyses the dismutation of the superoxide anion radical. SOD plays an important role in the defense against free radical damage in reperfusion injury and helps in reducing the infarct size during ischemia and reperfusion [12,13]. In the present study the serum SOD levels were decreased significantly in ischemic stroke cases decreased in large vessel infarcts than in small vessel infarcts) compared to controls.

Uric acid, most abundant endogenous aqueous antioxidant in humans, may protect against oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation during oxidative stress [14]. Several studies have shown that increased oxidative stress is associated with high circulating uric acid levels due to elevation of xanthine oxidase in stroke induced brain damage [15,16]. In this

study, there was a significant increase in the serum levels of uric acid in ischemic stroke cases (increased significantly in large vessel infarcts than in small vessel infarcts) than in controls. Serum TAC strongly correlated with serum uric acid. Under multivariate analysis, serum uric acid explained most of the variance in TAC during the study period.

This study suggests that estimation of serum vitamin E, vitamin C, SOD, uric acid and total antioxidant capacity may be used as an indirect evidence of oxidative stress induced neuronal damage in ischemic stroke.

The limitations of our study are as follows: We have not estimated any markers of lipid peroxidation such as malondialdehyde which along with antioxidant levels would better explain oxidative stress. Antioxidant levels were measured only once, but prospective serial estimations would better predict the antioxidant status with prognosis of stroke. This study was conducted on a small group of stroke patients. Larger clinical studies in this area are needed to establish the relationships between antioxidant capacity and oxidative damage following ischemia and reperfusion in man, and to form the basis of appropriate antioxidant intervention strategies to minimize long-term brain injury following cerebral ischemia.

COMPETING INTERESTS
None Declared

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REFERENCES

1. Leinonen JS, Ahonen JP, Lonnrot K, Jehkonen M, Dastidar P,Molnar G. Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke (2000). *Stroke* 31(9), 33-39.
2. Benzie FF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay (1996). *Analytical Biochem* 239, 70–76.
3. Baker H, Frank O (1969). *Clinical vitaminology*. Academic Press. New York, pp169–173.
4. Omage ST (1979). Ascorbic acid analysis II. Determination after derivatization with 2, 4, - Dinitrophenylhydrazine, *Methods in Enzymology*, 62, 7-8.
5. Nandi A, Chatterjee IB (1988). Assay of superoxide dismutase activity in animal tissues. *J Biosci*, 13 (3), 305-315.
6. Price CP, James DR (1988). Analytical reviews in clinical biochemistry: the measurement of urate. *Ann Clin Biochem*, 25 (5), 484-498.
7. Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. *Free Radical Biology and Medicine* (2005), 39 (7), 841-852.
8. Tornwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Albanes D, Huttunen JK (2004). Postintervention effect of alpha tocopherol and beta carotene on different strokes. A 6 year follow-up of the alpha tocopherol, beta carotene cancer prevention study. *Stroke*, 35(8), 1908-1913.
9. Yokoyama T, Date C, Kokubo Y, Yoshiike N, Matsumura Y, Tanaka H (2000). Serum vitamin C concentration was inversely associated with subsequent 20 year incidence of stroke in a Japanese Rural Community. *Stroke*, 31(10), 2287-2294.
10. Kurl S, Tuomainen TP, Laukkanen JA, Nyyssonen K, Lakka T, Sivenius J, Salonen JT (2002). Plasma vitamin C modifies the association between hypertension and risk of stroke. *Stroke*, 33(6), 1568-1573.
11. Joshi PP, Gawande AS, Ughade SN, Salkar RG (2003). Low plasma ascorbic acid in acute ischemic stroke. *Milestone*, 2(3), 119-125.
12. Kim GW, Kondo T, Noshita N, Chan PH (2002). Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia / reperfusion in mice. *Stroke*, 33(3), 809-815.
13. Zimmermann C, Winnefeld K, Streck S, Roskos M, Haberl RL (2004). Antioxidant Status in Acute Stroke Patients and Patients at Stroke Risk. *Eur Neurol*, 51(3), 157-161.
14. Weir CJ, Muir SW, Walters MR, Lees KR (2003). Serum urate as an independent predictor of poor outcome and future vascular events after acute stroke. *Stroke*, 34 (8), 1951-1957.
15. Hariklia VD, Apostolos IA, Haralambos IK (2008). The Role of Uric Acid in Stroke: The Issue Remains Unresolved**.** *Neurologist*, 14(4), 238-242.
16. Hozawa A, Folsom AR, Ibrahim H, Nieto JF, Rosamond WD, Shahar E (2006). Serum uric acid and risk of ischemic stroke : the ARIC study. *Atherosclerosis*, 187(2), 401-407.