

LETTER TO THE EDITOR

**OXIDATIVE DAMAGE AND PLASMA ANTIOXIDANTS
IN CEREBROVASCULAR ACCIDENT**

Sir,

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Cerebrovascular accident (CVA) is a leading cause of morbidity and mortality in major industrial countries. Growing evidence indicate that reactive oxygen species contribute significantly to this process, principally by means of lipid peroxidation (1). Oxidative stress is involved in the neuropathology of several other disorders like epilepsy, meningitis and Parkinson's disease (2, 3). Free radicals (viz., superoxide, nitricoxide, hydroxyl radicals) and other reactive oxygen species (viz., hydrogen peroxide, peroxy nitrite, hypochlorous acid) are produced in the body primarily as a result of aerobic metabolism. Antioxidants (glutathione, selenium, zinc, vitamins A, E, C) and antioxidant enzymes (superoxide dismutase, catalase, ceruloplasmin, glutathione reductase and peroxidase) exert synergistic actions in scavenging free radicals. Recent studies, primarily in animals, link oxidative stress to impaired endothelial function and vascular injury in atherosclerosis, acute hypertension, fluid percussion brain injury and ischemia (4). During ischemia, when oxygen supply is limited, calcium influx may activate phospholipase C that results in breakdown of membrane phospholipids, or may convert xanthine dehydrogenase to xanthine oxidase in the cerebral blood vessels leading to the formation of superoxide radicals and hydrogen peroxide. To evaluate the free radical metabolism in

CVA patients, plasma antioxidants viz., ceruloplasmin, vitamin A, vitamin E and vitamin C were estimated along with conjugated dienes as the lipid peroxidation marker.

Random blood samples were collected from the stroke patients meeting following criteria: Intra cerebral haemorrhage or cerebral thrombosis diagnosed by brain CT; No blood in ventricular system or subarachnoid space on brain CT; No evidence of organic heart disease or other possible cause of cerebral embolism. Cigarette smokers and alcoholics were excluded. The study population consisted of 46 patients with CVA, and 50 age and sex matched normal subjects. 46 patients included 16 cases (males 10, females 6) of intracerebral hemorrhage and 30 cases of cerebral thrombosis (males 21, females 9). All these patients were in an acute phase of stroke. Thus, the study included 3 groups hemorrhagic stroke, thrombotic stroke (ischemic stroke) and control group. 12 ischemic stroke patients who received aspirin (150 mg/day) or clopidogrel (75 mg/day) or warfarin (5 mg/day) were considered for follow up studies, after one month of treatment.

Random blood samples were collected in EDTA bottles from normal subjects and stroke patients. To estimate conjugated

TABLE I: Comparison of parameters in patients with CVA and controls (Mean±SEM).

<i>Parameters</i>	<i>Normal (n = 50)</i>	<i>Ischemic stroke (n = 30)</i>	<i>Hemorrhagic stroke (n = 16)</i>
Conjugated dienes (μ mol/dl)	10.26±1.42	11.58±1.2	11.2±1.38
Ceruloplasmin (mg/dl)	17.47±0.86	29.98±2.53 ^a	31.88±2.42 ^a
Vitamin A (μ g/dl)	70.14±6.3	62.56±10.08	68.63±13.91
Vitamin E (mg/dl)	10.56±0.69	7.59±0.78 ^b	7.38±0.75 ^c
Vitamin C (μ mol/L)	28.02±2.12	22.13±4.12	19.53±1.7 ^b

^aP<0.0001, ^bP<0.02, ^cP<0.05 significantly different from normal.

dienes, lipids were extracted from plasma with 2 : 1 chloroform methanol mixture, the extract was evaporated to dryness under a stream of oxygen-free nitrogen. The chloroform-free lipid was then redissolved in cyclohexane and absorbance at 234 nm was recorded against cyclohexane blank. Plasma ceruloplasmin was determined by its p-phenylene diamine (PPD) oxidase activity. Plasma α -tocopherol was measured using Emmorie Engel reaction which is based on the reduction by tocopherols of ferric to ferrous ions which then forms a red complex with α - α dipyridyl. Vitamin A has an absorption maximum in the UV region of 327 m μ and can be determined by reading the extinction at this wavelength before and after exposure to UV light, to destroy the vitamin A present. Ascorbic acid is oxidised by copper to form dehydroascorbic acid which then reacts with acidic 2, 4 dinitro phenyl hydrazine to form red bishydrazone. The intensity of red colour is measured at 520 nm. Data were analyzed statistically by Mann-Whitney U test and Wilcoxon rank sum test. The difference of P<0.05 were considered significant. Mean plasma conjugated dienes was slightly higher

in case of ischemic stroke patients as compared to the normal subjects (Table I). However normal value was obtained in these patients after treatment (Table II). Plasma ceruloplasmin level was significantly high both in pretreatment and post-treatment cases of ischemic stroke compared to controls. Vitamin E was significantly low in ischemic stroke patients compared to normal. Though the vitamin A and C levels decreased in ischemic stroke patients compared to controls, the decrease was statistically insignificant (Table I). There

TABLE II: Comparison of parameters in patients with ischemic stroke before treatment and after treatment (Mean±SEM).

<i>Parameters</i>	<i>Pretreatment (n = 12)</i>	<i>Posttreatment (n = 12)</i>
Conjugated dienes (μ mol/dl)	11.54±1.96	10.36±1.11
Ceruloplasmin (mg/dl)	27.71±3.06	26.53±2.63 ^a
Vitamin A (μ g/dl)	60.25±9.85	65.33±5.78
Vitamin E (mg/dl)	8.63±1.19	10.93±0.94
Vitamin C (μ mol/L)	21.03±4.16	22.96±2.76

^aP<0.01 significantly different from normal.

was an apparent increase in conjugated dienes in hemorrhagic stroke patients compared to normal subjects. Further, ceruloplasmin increased significantly in these patients. No significant difference was observed in vitamin A levels, however vitamin E and C levels decreased significantly in these patients as compared to controls (Table I).

Experimental studies on animals indicated that there is a relationship between free radical production and increased lipid peroxidation in the brain during CNS ischemia or trauma (1). Oxidative stress has been associated with the development of blood brain disruption and cellular injury after ischemia in mice (4). Mean conjugated dienes, the initial product of lipid peroxidation was high in CVA patients as compared to normal individuals in the present study. This fact is supported by earlier reports which indicate significantly high serum malondialdehyde, the end product of lipid peroxidation, in ischemic stroke (5). High lipoperoxides in erythrocytes is the potential risk factor for cerebral hemorrhagic stroke in humans (6). These findings are in keeping with possible evidence of free radical production and damage in CVA. In both ischemic stroke and hemorrhagic stroke, ceruloplasmin activity in plasma is significantly high compared to healthy subjects. Ceruloplasmin, through its ferroxidase activity, may decrease the availability of Fe^{+2} and Cu^{+1} for Haber-weiss and Fenton reaction that generate free radicals and thus protect the patients from aggravating free radical injury. Vitamin A levels in stroke patients remained low compared to controls in the present study.

One of the earlier reports also indicates significantly low plasma carotenoids in acute ischemic stroke (7). In the present study, vitamin E concentration is significantly reduced in both ischemic stroke and hemorrhagic stroke. Ryglewicz et al (8) observed that a low concentration of antioxidants such as alpha tocopherol may influence the development of post stroke dementia. Likewise, deficiency of vitamin C causes insufficiently hydroxylated collagen giving rise to poor wound healing and fragility of blood vessels. Ascorbic acid is an important antioxidant which may help to reduce free radical damage and atheroma formation in blood vessels. Serum ascorbate level and total antioxidant capacity of stroke patients decreased after the onset of ischemia (9). A similar finding has been observed in the present study also. Low plasma vitamin C was associated with increased risk of stroke, especially among hypertensive and over-weight men (10). Ascorbic acid and α -tocopherol have antioxidant properties that could improve redox sensitive vascular changes associated with hypertension in stroke prone spontaneously hypertensive rats (11). It is a well known fact that "sparing action" of ascorbic acid on vitamin E is linked to its ability to reduce tocopheroxyl radicals formed by scavenging of other free radicals. This explains the cause for the decrease in plasma vitamin E in the present study.

Measurements of various parameters were made in 12 ischemic stroke patients who were treated with aspirin and mannitol and who showed symptomatic recovery. Mannitol, a water soluble antioxidant is supposed to prevent ascorbate auto-oxidation and ascorbate dependent hydroxyl

ion formation (12). In the present study, the drugs given to the ischemic stroke patients probably reacted with the free radicals, as indicated by decreased conjugated dienes with concomitant increase in antioxidant vitamins.

On the whole, it can be concluded that the plasma antioxidant defense capacity was low in acute phase of stroke, perhaps as a result of increased oxidative damage, and the condition tends to reverse to normal after treatment.

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REFERENCES

1. Kocaturk PA, Akbostanci MC, Ical A, Tuncel D, Kavay GO, Mutluer N. Antioxidant status in cerebrovascular accident. *Biol Trace Elem Res* 2001; 80(2): 115-124.
2. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. *Clin Chim Acta* 2001; 303: 19-24.
3. Sudha K, Rao AV, Rao SN, Rao A. Free radical toxicity and antioxidants in Parkinsons disease. *Neurol India* 2003; 51(1): 60-62.
4. Kim GW, Lewen A, Copin J, Watson BD, Chan PH. The cytosolic antioxidant, copper/zinc superoxidase dismutase attenuates blood brain barrier disruption and oxidative cellular injury after photo thrombotic cortical ischemia in mice. *Neuroscience* 2001; 105(4): 1007-1018.
5. Gariballa SE, Hutchin TP, Sinclair AJ. Antioxidant capacity after acute ischemic stroke. *QMJ* 2002; 95(10): 691-693.
6. Chen HH, Zhou JF. Low cholesterol in erythrocyte membranes and high lipoperoxides in erythrocytes are the potential risk factors for cerebral hemorrhagic stroke in human. *Biomed Environ Sci* 2001; 14(3): 189-198.
7. Polidori MC, Cherubini A, Stahl W, Senin U, Sies H, Mecocci P. Plasma carotenoid and malondialdehyde in ischemic stroke patients: relationship to early outcome. *Free Radic Res* 2002; 36(3): 265-268.
8. Ryglewicz D, Rodo M, Kunicki PK, Bednarska Makaruk M, Graban A, Lojkowska W, Wehr M. Plasma antioxidant capacity and vascular dementia. *J Neurol Sci* 2002; 15: (203-204): 195-197.
9. Sharpe PC, Mulholland C, Trinick T. Ascorbate and malondialdehyde in stroke patients. *Ir J Med Sci* 1994; 163: 488-491.
10. Kurl S, Tuomainen TP, Laukkanen JA, Nyyssonen K, Lakka T, Sivenius J, Salonen JT. Plasma vitamin C modifies the association between hypertension and risk of stroke. *Stroke* 2002; 33(6): 1568-1573.
11. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant effects of vitamin C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* 2001; 38: 606-611.
12. Prabhu HR, Krishnamurthy S. Ascorbate dependent formation of hydroxyl radicals in the presence of iron chelates. *Ind J Biochem Biophys* 1993; 30: 289-292.