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Prognostic significance of serum free radical level in head injury

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Abstract: There is evidence that free radicals are released following head injury. Serial estimation of free radical levels in serum of isolated head injury cases showed high levels on the day of injury. Cases having parenchyma damage i.e. diffuse axonal injury and brain edema showed significantly high levels until seventh day of injury whereas cases in whom surface hematoma i.e. extradural hematoma was evacuated showed decline of free radical level to normal. Free radical level is reflected in serum possibly due to breakdown brain blood barrier.

Keywords: blood-brain barrier. brain edema, free radicals, head injury prognosis, intracranial hematoma,

INTRODUCTION

Nitric oxide is a reactive nitrogen species that has been linked to excitotoxicity. It reacts with the superoxide radical to form peroxynitrite, which causes lipid peroxidation, DNA damage, and protein oxidation. Nitric oxide is a potent vasodilator and also acts as neurotransmitter. Raised level of nitric oxide in plasma has been reported in Alzheimers disease, amyotrophic lateral sclerosis and inflammatory brain diseases. Serial study of nitric oxide in head injury patients with an objective to correlate prognosis have not been reported.

MATERIAL AND METHODS

Ninety seven patients of acute head injury and 68 healthy voluntary donors with comparable age and sex groups were selected for this study. Level of consciousness and C.T.Scan findings were recorded. Polytrauma cases were not included. Since nitric oxide is oxidised to nitrite, estimation of the nitrite levels was performed by method of Ding et al[1]. Plasma nitrite levels were estimated on Day-0, Day-4 and Day-7. Normal plasma nitrite level averages from 16 to 62 µmol/L with a mean value of 28.9µmol/L[2].

OBSERVATIONS

This study comprised of 97 head injury patients with age ranging from 4 years to 74 years (average age : 29.6 years) with peak incidence in second and third decade of life (43.4%). The Male:Female ratio was 3:1. Control was taken from 68 healthy voluntary donors with an average age of 31.2 years (ranges from 6 years to 52 years) and their mean plasma nitrite level was 31.59±11.27 µmol/litre. Vast majority of head injury cases were due to traffic accident or fall from height (47.4% and 38.1%) respectively. Eighty one percent had Glasgow Coma Score(GCS) more than 8 and 19% had severe injury (GCS <8) . CT was done in all cases at the time of admission revealing intracranial lesions were noted (Table 1). Forty five percent had evidence of cerebral edema as low attenuation areas, after associated with areas of contusion. In cases with coma score more than 8, nitrite levels did not show significant change as compared to control whereas in cases with severe head injury (GCS <8), there was significant rise in plasma nitrite levels (Table 2). Fifty three patients having no brain edema revealed a lower level of plasma nitrite as compared to 44 patients who had cerebral edema on CT (Table 3). Significant difference was also noted in cases of diffuse axonal injury, intracerebral haematoma and acute subdural hematoma but it was not significant in EDH group (Table 4). Cases having recovery showed a decline of plasma nitrite levels to normal level by 7 days whereas those cases who later expired, the plasma nitrite levels remained persistently high.

Table 1. Distribution of Patients According to Pathology.

Pathology	Patients (n=97)	Percentage (%)
EDH	36	37.11
ICH	44	45.36
SDH	11	11.34
DAI	06	6.19
Control (n=68)	68	100

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Table 2. Nitrite level (µmol/L) in Patients with GCS >8 and < 8

Plasma Nitrite Level in control group (n=68)	Plasma Nitrite Level in GCS >8 patients (n=69)			Plasma Nitrite Level in GCS<8 patients (n=28)		
	Day 0	Day 4	Day 7	Day 0	Day 4	Day 7
Mean 31.59	51.7	53.84	50.88	77.18	75.89	68.22
SD : ± 11.27	±31.29	±30.20	±27.13	±37.08	±36.34	±32.52
q value	5.54	4.92	4.26	8.70	8.69	7.93
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3. Nitrite level (µmol/L) in Patients with Brain Edema and Without Brain Edema

Plasma Nitrite Level in control group (n=68)	Plasma Nitrite Level in without Brain Edema (n=53)			Plasma Nitrite Level in Brain Edema (n=44)		
	Day 0	Day 4	Day 7	Day 0	Day 4	Day 7
Mean 31.59	50.42	50.96	48.96	69.46	71.34	64.14
SD : ± 11.27	±30.07	±29.06	±25.22	±37.64	±35.39	±32.67
q value	4.09	4.42	4.40	7.92	8.72	7.88
p value	<0.01	<0.01	<0.01	<0.001	<0.001	<0.001

Table 4. q value of Students Newman-Keuls test (SNK-test) and their significance in inter group comparison.

Comparison Groups	day 0		day 4		day 7	
	q value	p value	q value	p value	q value	p value
DAI vs. control	9.65	<0.001	8.85	<0.001	10.15	<0.001
DAI vs. EDH	8.18	<0.001	7.63	<0.001	8.92	<0.001
DAI vs. SDH	6.19	<0.001	4.64	<0.01	6.45	<0.001
DAI vs. ICH	6.16	<0.001	4.67	<0.01	5.32	<0.001
ICH vs. control	7.53	<0.001	8.91	<0.001	5.73	<0.001
ICH vs. EDH	4.43	<0.01	6.40	<0.001	4.04	<0.05
ICH vs. SDH	1.28	NS	0.88	NS	0.54	NS
SDH vs. control	3.65	<0.05	5.00	<0.01	7.87	<0.001
SDH vs. EDH	1.65	NS	3.31	<0.05	5.36	<0.001
EDH vs. control	3.01	<0.05	2.50	NS	2.49	NS

DISCUSSION

Oxidative stress describes the potential for free radical damage that exists in metabolically active tissues. Free radicals are generated during normal metabolism and a number of systems are in place to prevent the potential damage that can occur from oxidation of lipids, proteins, and DNA. Because of the high metabolic rate in the brain, a substantial amount of free radicals are continually generated, which mandates a generous antioxidant reserve. Depletion of these reserves can lead to oxidative stress and tissue damage by free radicals.

TBI initiates a number of processes that result in increased free radical production, including excitotoxicity, inflammation, and electron transport chain dysfunction. The free radical molecules that are important potential mediators of secondary injury following TBI can be classified as reactive oxygen or reactive nitrogen species. The superoxide radical ($O^{\cdot}_{2}$) is converted to hydrogen peroxide through the action of superoxide dismutase (SOD). Both are reactive oxygen species that create highly reactive hydroxyl radicals (OH) in the presence of transitional metals, such as iron and copper. Hydroxyl radicals react with all biomolecules, so their potential for tissue damage is great.

Endogenous antioxidants exist to prevent the tissue damage that can result 'from free radical formation. The antioxidant enzymes that are important in the CNS are SOD, catalase, and glutathione peroxidase. The action of SOD is described previously, converting superoxide to hydrogen peroxide. Catalase then converts hydrogen peroxide to water and oxygen. Glutathione peroxidase can also metabolize hydrogen peroxide, in addition to peroxidated lipids. There are also antioxidant reserves that consist of the low-molecular-weight antioxidants glutathione, ascorbate, and tocopherol. Glutathione either serves as a substrate in the reactions mediated by glutathione peroxidase, or independently reacts with free radicals. Ascorbate and tocopherol can work in conjunction with each other. Tocopherol reacts with lipid radicals to prevent lipid peroxidation, and is then recycled by ascorbate. Ascorbate also scavenges numerous free radicals independently and is present in CSF in concentrations approximately 10 times higher than in plasma[3]. Interestingly, ascorbate also has pro-oxidant properties in the presence of free transitional metals.

A wealth of data exists to support the important role of oxidative stress in secondary injury after head trauma. Hydroxyl radical formation has been demonstrated following experimental closed-head injury in rats, and nitrite and nitrate concentrations (the oxidation products of nitric oxide) are increased in the CSF of humans following TBI[4]. Increased catalase and glutathione peroxidase activities have been found in rats following TBI[5], whereas total antioxidant reserves are decreased and lipid peroxidation products are increased [6].

There is also experimental evidence that supports a role for oxidative stress specifically in shaken baby syndrome[7]. Six-day-old rats were subjected to daily shaking for 3 days using a device that replicates the mechanical acceleration deceleration injury seen in human infants that are shaken. Hydroxyl radical was increased 1 hour following the third episode of shaking, which was significantly attenuated by antioxidant therapy with tirilazad. Antioxidant treatment also decreased the amount of hemorrhage associated with

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shaking, but did not impact the loss of cortical tissue measured 1 and 2 weeks after injury. Treatment with tirilazad also improved the effect of antiexcitotoxic therapy in this experimental model[8].

Oxidative stress has been demonstrated in children following TBI[9]. F2 isoprostane, a marker of lipid peroxidation, is increased fourfold in the CSF of children following head injury compared with control, indicating free radical damage to membrane lipids. Also, the antioxidant reserve of the CSF in these children was decreased, indicating a depletion of the normal antioxidative mechanisms present in the brain. Specifically, ascorbate levels in the CSF following head traumat were much lower than in control samples, with a corresponding increase in ascorbate radical, again supporting a role for ascorbate as a key antioxidant in the brain.

Similar to antiexcitotoxic therapies, clinical trials using antioxidants after TBI in adults have been unsuccessful. the two most prominent clinical trials evaluated the use of polythylene glycol SOD and tirilazad[8]. Despite the effort to make superoxide dismutase more permeable to the blood-brain barrier and increase its half-life by conjugation with polythylene glycol, the ability of both of these substances to enter the brain is limited. Also, no measurement of oxidative stress was attempted in the patients enrolled in the study, thereby allowing a potentially heterogeneous group. With the current tools available to quantitate, at least in part, oxidative stress in individual patients, further antioxidant therapies may be more successful. Trials of antioxidant therapeutic strategies is needed.

CONCLUSION

The level of nitric oxide increases in head injury patients and decreasing level was observed with recovery of the patients. The levels were high in those patients with severe cerebral injury and remained high in those patients who had fatal outcome.

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