OXIDANTS AND ANTIOXIDANTS IN HYPOXIC ISCHAEMIC ENCEPHALOPATHY

V.N. Thorat*, A.N. Suryakar*, A.S. Sardeshmukh* and S.S. Sarawade**

*Department of Biochemistry, Dr. V. M. Medical College, Solapur.

**Department of Paediatrics, Govt. Medical College, Nanded.

ABSTRACT

In the present study, the role of serum lipid peroxide and serum nitric oxide as oxidants and erythrocytic superoxide dismutase & serum vitamin E as antioxidants were determined in the 50 neonates with hypoxic ischaemic encephalopathy (HIE) as against 25 healthy neonates as controls. 50 patients of HIE were further divided into two groups i.e. mild and moderate HIE patients. All subjects were in the age group of 37-41 weeks of gestation. The levels of serum lipid peroxide, serum nitric oxide and erythrocytic superoxide dismutase were significantly elevated in both groups of neonates with HIE than those of controls ($P < 0.001$), whereas serum vitamin E levels were significantly decreased in both groups of HIE patients than those of controls ($P < 0.001$). A positive correlation was obtained between serum lipid peroxide and erythrocytic superoxide dismutase ($r = +0.86$). Alterations in the status of oxidants and antioxidants indicate role of free radicals in the development of HIE.

KEY WORDS

Lipid peroxide, nitric oxide, superoxide dismutase, vitamin E, hypoxic ischaemic encephalopathy.

INTRODUCTION

The neurology of the newborn is a topic of major importance because of the pre-eminence of neurological disorders in neonatology (1).

Encephalopathy is a term used to describe central nervous system dysfunction. Neonatal encephalopathy associated with perinatal asphyxia is called hypoxic ischaemic encephalopathy. Free radicals are generated during hypoxic ischaemic encephalopathy within mitochondria & also as by-products in the synthesis of prostaglandins. These cause brain damage by attacking membranous fatty acids (2, 3, 4, 5).

Nitric oxide (NO) mediates, in part, the cytotoxic activity of macrophages and blood vessel relaxation produced by endothelial cells and it also acts as a neurotransmitter in the central and peripheral nervous system, so the nitric oxide (NO) is involved in the cascade of metabolic events that causes or contributes HIE (6).

The antioxidants play an important role in reducing oxidative stress but the brain is relatively

insufficiently protected by antioxidants against the free radical induced damage (7).

Nitric oxide and free radicals are involved at different stages of asphyxia. This stimulates interest in the development of strategies capable of preventing or limiting the expression of this injury. In view of complexity, HIE still remains to be a puzzle for the neurologists and neonatologists with regard to the pathophysiology, type and timing of intervention. It has crucial importance to increase the understanding of the events leading to brain injury.

In view of the above concepts the present study has been planned to assess the role of erythrocytic superoxide dismutase, serum vitamin E, serum nitric oxide & serum lipid peroxide during hypoxic ischaemic encephalopathy (HIE) in comparison to control neonates.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Dr. V. M. Medical College, Solapur and Shree Chhatrapati Shivaji Maharaj General Hospital, Solapur during Aug 2001 to Dec. 2002.

Present study included 25 neonates with mild HIE and 25 neonates with moderate HIE with birth asphyxia (Apgarscore 4 or less at 1 minute of life)

Author for correspondence :

Dr. A. N. Suryakar
Prof. & Head,
Department of Biochemistry,
Dr. V. M. Medical College, Solapur, INDIA

and surviving for 24 hrs. or more and 25 normal term newborns as controls. All neonates in the study were of gestational age 37-41 weeks. Staging of HIE was done according to the criteria of Sarnat and Sarnat (8). All the HIE patients were put on oxygen just after delivery while 25 normal controls had unevenful stay in the hospital. This study was approved by the ethical committee of hospital.

5 ml venous blood was collected at 24 hrs. after birth. 1 ml. blood was taken in EDTA and used for the determination of erythrocytic superoxide dismutase activity by the method of Marklund & Marklund (9). Remaining blood sample was used for the assessment of serum lipid peroxide as malondialdehyde (MDA) by Satoh's method (10), serum nitric oxide as nitrite (NO) by the method of Cortas & Wakid (11) and serum vitamin E by the method of Baker and Frank (12).

Statistical analysis was carried out using students paired & unpaired 't' test. P value less than 0.05 ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

Significant increase in serum lipid peroxide (MDA) and serum nitric oxide (NO) were observed in both groups of HIE patients as compared to controls ($P < 0.001$). Further it was noted that the serum lipid peroxide and serum nitric oxide levels were significantly increased in moderate HIE than those of mild HIE patients ($P < 0.001$). During the asphyxiated insult arachidonic acid production increases and after reperfusion it is metabolized to vasospastic substances, thus utilizing prostaglandin endoperoxidase synthase activity which in turn produced excess free radicals (13).

The most commonly implicated mechanism for generation of oxygen free radicals in asphyxia is hypoxanthine / xanthine oxidase system. Hypoxanthine accumulates in the tissues, plasma & other body fluids during hypoxia. When high concentration of oxygen is administered to hypoxic patient during reperfusion / resuscitation, large amount of oxygen free radicals are produced (14, 15, 16).

The results of present study are in agreement with earlier reports (13, 14, 15, 16, 17).

Thiringer *et al.* and McCord J.M. *et al.* in their studies demonstrated that oxygen free radicals were generated during reperfusion/ reventilation stage and free radical scavengers and calcium antagonists had beneficial effects (18, 19).

The brain is especially rich in polyunsaturated phospholipids and accordingly, is susceptible to free

radical attack. Free iron and nitric oxide are important contributory to oxidative injury. They transform mildly reactive oxygen species to more toxic free radicals thereby leading to increased level of lipid peroxidation (2).

Yuan Shi *et al.* reported that nitric oxide levels after perinatal asphyxia were related to the severity of neonatal HIE and brain damage (20).

This is parallel with the results obtained in the present study.

The levels of NO were increased in moderate HIE than those of mild HIE ($P < 0.001$). Iadecola has shown that during the mild stage of asphyxia, endothelial NOS (e-NOS) is activated in the brain for cerebral protection by increasing cerebral blood flow. Neuronal NOS (n-NOS) is also activated shortly after the asphyxial event and is known to cause increase in the neuronal NO which produces several neurotoxic effects. After the first 12 - 24 hrs of asphyxia, inducible NOS (i-NOS) in the glial cells becomes activated causing large increases in cerebral NO which is now considered to have a major role in the ongoing neurotoxicity following asphyxia (21).

NO has emerged as an important neuronal messenger in both the central and peripheral nervous systems. Models of the potential diffusion of NO suggest that even with brief bursts of NO (100 msec) and a short half - life (0.5-5sec) the physiologic sphere of influence could extend as far as 10 μ m from the point of origin. NO has been implicated in a variety of functions including the nonadrenergic, non-cholinergic (NANC) neurotransmission in several peripheral systems, in the control of local regulation of cerebral blood flow and in the control of synaptic plasticity & sensory signaling. (22)

Elevation in the level of erythrocytic superoxide dismutase (SOD) activity in HIE patients in the present study may be due to production of superoxide and hydrogen peroxide in the brain injury cases and subsequent upregulation of antioxidant enzyme (SOD). But the increased activities of these enzymes were unable to scavenge the free radicals. Oxygen should be administered to the cases of HIE only if it is needed to maintain normal blood gas levels. Liberal use of oxygen may increase asphyxia related injury by producing oxygen free radicals (17).

Depletion in the levels of serum vitamin E in HIE patients in the present study is attributed to following mechanism.

Peroxy radicals are generated in the chain reaction

Table 1. Shows concentrations of oxidants and antioxidants in controls and HIE patients

Sr. No.	Serum lipid peroxide (nmol MDA/ml)	Serum nitric oxide (μ mol nitrite /L)	Erythrocytic SOD (U/gm Hb)	Serum Vitamin E mg/L
Controls (n=25)	2.61 $\pm$ 0.815	33.7 $\pm$ 8.87	216.52 $\pm$ 61.73	4.54 $\pm$ 1.32
HIE (n=50)	6.571 $\pm$ 1.87	86.47 $\pm$ 20.66	735 $\pm$ 102.62	1.59 $\pm$ 6.61
Mild HIE (n=25)	4.75 $\pm$ 0.493**	70.33 $\pm$ 11.27**	595 $\pm$ 109.85**	2.03 $\pm$ 0.52**
Moderate HIE (n=25)	8.40 $\pm$ 0.68 *	101.01 $\pm$ 17.27*	876 $\pm$ 87.65*	1.15 $\pm$ 0.27*
n = number of subjects studied values are expressed as mean $\pm$ S.D. ** p<0.001 as compared to controls. *p<0.001 group 2A versus Group 2B				

of lipid peroxidation which is self-perpetuating particularly in the brain with high concentrations of unsaturated lipids (PUFAs) - unless the reaction is quenched by a chain breaker. α -tocopherol is the most effective chain breaker in cellular membranes & in lipid phases & thus play a significant role as an effective antioxidant (2).

In the present study positive correlation was obtained between serum lipid peroxide & erythrocytic SOD ($r = + 0.86$).

Alterations in the status of oxidants and antioxidants indicate a pivotal role of free radicals in the development of HIE.

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Note: $r = +0.86$

Fig. 1. Showing correlation between serum lipid peroxide and erythrocytic SOD in HIE patients

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