Status of oxidant and antioxidants in term newborns with hypoxic ischemic encephalopathy: A case control design

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Abstract— *Hypoxic ischemic encephalopathy (HIE) is due to failure to initiate and sustain breathing immediately after delivery which may leads to increase mortality and morbidity in these neonates. This may cause severe and permanent neuropsychological sequelae, including mental retardation, visual motor or visual perceptive dysfunction, increased hyperactivity, cerebral palsy and epilepsy. The brain damage may occur due to release of free radicals in HIE. So this case control study was conducted on 50 neonates with HIE and 25 healthy neonates with the aim to compare the status of oxidants and antioxidants in these both groups. It was observed that Malonyldialdehyde (MDA) was found with significantly increased in study group than control group i.e. 12.26±4.1 nmol/ml in study and 2.10±0.10 nmol/ml in control group. Among antioxidants, Catalase (30.42± 7.7 v/s 14.59± 4.7) and SOD (2.59± 0.4 v/s 1.54± 0.3) were found significantly increased whereas Vit E (1.36± 0.3 v/s 2.73± 0.6) was found significantly decreased in neonates with HIE than controls.*

Keywords: Hypoxic ischemic encephalopathy (HIE), Malonyldialdehyde (MDA), Superoxide dismutase (SOD), Catalase and Vitamin E.

I. INTRODUCTION

Failure to initiate and sustain breathing immediately after delivery has been associated with hypoxic ischemic injury to the central nervous system (CNS) and the clinical manifestations of this injury have been termed as hypoxic ischemic encephalopathy (HIE). In India 9% of inborn babies has Apgar score of <7 at 1 minute and 1.5% of those suffer from Hypoxic ischemic encephalopathy (HIE).[1]

Perinatal hypoxic-ischemic encephalopathy (HIE) occurs in one to three per 1000 live full-term births.[2] Of affected newborns, 15%–20% die in the postnatal period and an additional 25% develop severe and permanent neuropsychological sequelae, including mental retardation, visual motor or visual perceptive dysfunction, increased hyperactivity, cerebral palsy, and epilepsy.[3]

In HIE, free radicals are generated within mitochondria and also as by products in the synthesis of prostaglandins. These cause brain damage by attacking membranal fatty acids mainly polyunsaturated fatty acids (PUFA) and this is indicated by elevated levels of malonyldialdehyde (MDA). This process of damage to PUFA is knows as lipid peroxidation.[4]

Along with this, reduced antioxidative capacities of neonates may contribute to the pathogenesis of disorders in the perinatal period. Superoxide dismutase (SOD), Catalase and Vitamin E are three important antioxidants which help in protecting biological structures from free radical mediated injury.[5]

So this present study was conducted to elucidate the alterations in the expressions of superoxide dismutase(SOD), catalase, vitamin E and the levels of malonyldialdehyde as an index of lipid peroxidation (LPO) during HIE in comparison to control neonates.

II. METHODOLOGY

This case control study was conducted in the NICU of Department of Pediatrics in collaboration with Department of Biochemistry at Era's Lucknow Medical College and Hospital, Lucknow (U.P.) India, from January 2014 to June 2015.

Study was conducted on 75 full term neonates i.e. 37-41 weeks of gestation of women delivered in gynecology department of Era's Lucknow Medical College and Hospital, Lucknow. Out of these 75 neonates, 50 neonates were with Hypoxic ischemic encephalopathy (HIE) as study group and 25 neonates were healthy controls were excluded from the study.

HIE Failure to initiate and sustain breathing immediately after delivery had been associated with hypoxic-ischemic injury to the CNS and the clinical manifestations of this injury have been termed as HIE.[1] Clinical manifestations were defined by Sarnat & Sarnat staging.[6] were excluded from the study.

The New Ballard Score (NBS)[7] was used to estimate gestational age (GA) in all the cases and controls newborn within 48 hours of birth.

Birth asphyxia[8] was defined as requirement for positive pressure ventilation including bag and mask ventilation or intubations for more than 1 minute during postnatal resuscitation and an Apgar score of 6 or less at 5 minutes. Progression of birth asphyxia to HIE was defined as presence of minimum of 2 abnormal neurological findings out of the following as alteration of muscle tone either hypotonia / hypertonia, abnormal neonatal reflexes including the Moro's, rooting and sucking, failure to arouse the newborn even after vigorous stimulation, presence of convulsion.

After written informed consent was obtained from parents, demographic and clinical information through maternal interviews and medical record reviews was collected of study participants.

After obtaining prior consent from the parents, 3 ml venous blood were collected from the subjects at 24 hrs after birth in EDTA vials under aseptic condition by venipuncture using 5 ml disposable syringe needle. Serum and plasma were separated by centrifugation at 3000 rpm for 10 min. at room temperature. Plasma was used for estimation of MDA whereas RBCs were lysed by mixing chilled water and RBC lysate was used for the estimation of antioxidants. The samples were stored at -20ºC before analysis and all the samples were analyzed on the same day of collection.

Oxidants and Anti-oxidants were estimated and expressed in mean±SD and clinical & demographic data were expressed in percentage and proportions. Statistical analysis was done by using student's 't'-test, Gaussian z-test, fisher exact test. Chi-square test was used for categorical comparisons. To correlate levels of oxidant and antioxidants with severity & staging of HIE one way ANOVA test was used. $p<0.05$ was considered as significant.

III. RESULT

Out of total 75 participants, 50 neonates were with Hypoxic ischemic encephalopathy (HIE) as study group and 25 neonates were healthy controls were excluded from the study.

Mean gestational age of case and control group neonates was 38.44±1.3 weeks and 38.54±1.3 weeks respectively, while mean birth weight of case and control group neonates was 2.61±1.3 and 2.85±1.7 respectively. Both the groups were found comparable ($p>0.05$) as per gestational age and weight of newborn. (Table 1)

As per the gender of participants, 38 (76%) males and 12 (24%) females neonates in study group were evaluated against 16 (64%) males & 9 (36%) females neonates included in the control group. Gender wise these two groups were also comparable. (Table 1)

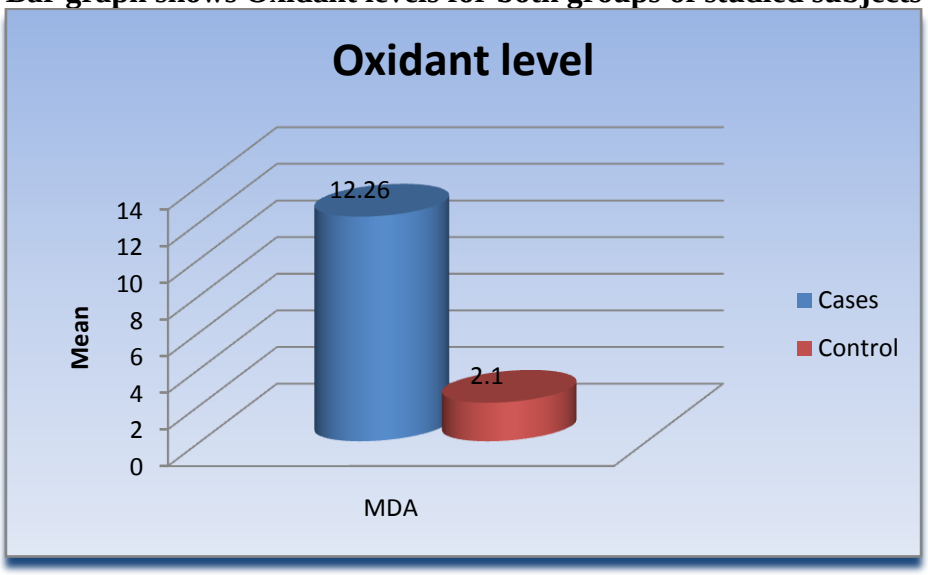
Table 1
Comparison of demographic variables of Study and Control Groups

S. No.	Demographic Variables	Study Groups (N=50)	Control Groups (N=25)	P value
1	**Gestational Age (Mean±SD Weeks)**	38.54±1.3	38.44±1.3	**0.757***
2	**Birth Weight (Mean±SD Kg)**	2.61±1.3	2.85±1.7	**0.499***
3	**Male: Female**	38:12	16:9	**0.289****

***Unpaired 't' test** ***Chi square test**

Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (MDA). This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells. While in study group its mean value was found to be 12.26±4.1 nmol/ml, among controls the value was 2.10±0.10 nmol/ml only. The variation between the two values of MDA was highly significant, P<0.001. (Figure 1)

Figure 1
Bar graph shows Oxidant levels for both groups of studied subjects



Mean of antioxidant enzymes catalase, among cases were 30.42±7.7µmol H2O2/min/mg, Vitamin E 1.36±0.3mg/dl, and enzymes superoxide dismutase (SOD) 2.59±0.4units/ml, the corresponding quantities for controls were 14.59±4.7 µmol H2O2/min/mg, 2.73±0.6mg/dl and 1.54±0.3units/ml respectively. The differences in the magnitude of these three antioxidant between the two categories were highly significant, P<0.001. (Table2)

Table 2
Comparison of Antioxidant Status in Study and Control Groups

S. No.	Antioxidants Status	Study Groups (N=50)	Control Groups (N=25)	P value
1	**Catalase (nmol H_2O_2/min/mg)**	30.42± 7.7	14.59± 4.7	**<0.001***
2	**Vit E (mg/dl)**	1.36± 0.3	2.73± 0.6	**<0.001***
3	**SOD (units/ml)**	2.59± 0.4	1.54± 0.3	**<0.001***

***Unpaired 't' test**

The mean Oxidant level of MDA was 11.06±1.7 nmol/ml in stage I, 11.44±1.6 nmol/ml in the stage II and 14.67±7.8 nmol/ml in the stage of HIE III among newborn patients. As for the antioxidant enzymes Catalase, their corresponding magnitudes for three categories were 27.12±6.5 µmol H_2O_2/min/mg, 31.45±7.6 µmol H_2O_2/min/mg and 32.13±8.1 µmol H_2O_2/min/mg respectively. Similarly values of Vitamin E for three stages were 1.75±0.2 mg/dl, 1.29±0.3 mg/dl, and 1.26±0.3 mg/dl respectively. Further, magnitude of enzymes superoxide dismutase (SOD) in respect of each of above category of HIE were 2.10±1.2 units/ml, 2.56±1.8 units/ml and 2.86±1.9 units/ml. The difference in these values among the three stages of HIE in respect to Oxidant as well as antioxidant levels, MDA, enzyme Catalase and Vitamin E were highly statistically significant, P<0.001. (Table 3)

Table 3
Comparison of Oxidants and Antioxidant Status in various stages of HIE

Oxidants and Antioxidants Status		Stages of HIE			P value
		Stage I (N=24)	Stage II (N=17)	Stage III (N=9)	
Oxidants	**MDA (nmol/ml)**	11.06±1.7	11.44±1.6	14.67±7.8	
Antioxidants	**Catalase (nmol H_2O_2/min/mg)**	27.12±6.5	31.45± 7.6	32.13± 8.1	**<0.001***
	Vit E (mg/dl)	1.75±0.3	1.29± 0.3	1.26± 0.2	**<0.001***
	SOD (units/ml)	2.10±1.2	2.56± 1.8	2.86 ± 1.9	**<0.001***

**** ANOVA Test***

IV. DISCUSSION

This present study significant increase in serum lipid peroxide (MDA) was observed in HIE patients as compared to controls (P<0.001). These observations were well in resonance to the study conducted by V.N. Thorat et al[4]. Singh S K et al**Error! Bookmark not defined.** also reported MDA was significantly higher in cases as compared to the controls. The results of present study are also in agreement with earlier studies like studies done by Palmer, C et al., Saughstad, O.D. et al. and Wolfe, L.S. et al.[10, 11,12] 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. Hypoxanthine accumulates in the tissues, plasma and 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.[10,11]

The antioxidants which counterattack oxygen free radicals include enzymes like Superoxide dismutase, Catalase and non-enzymatic vitamins like vitamin E and elements like Zn.

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 as reported by Wolfe L.S.[12]

In this study the level of SOD and catalase were significantly high in HIE group than controls (P<0.001). The present study was showing the similar results to the study done by Singh S K et al**Error! Bookmark not defined.** in 1999.This may be due to production of O_2^- and H_2O_2 in HIE cases and subsequent upregulation of antioxidant enzymes SOD and catalase. The increased level of MDA

indicates that the upregulation of SOD and catalase was not able to prevent lipid peroxidation by oxygen free radicals. Ueda et al[13] found insignificant variations in red cell SOD activity of new born infants. Autor et al[14] observed significantly lowered red cell SOD activity in newborns when compared to adults. However, Bonta et al[15] demonstrated an inverse relationship between red cell SOD activity and gestational age and showed lower SOD levels. Ray GN et al[16] and Batra S et al[17] reports on changes in free radicals in both asphyxia and hypoxic ischemic encephalopathy have shown increased level of SOD, O_2^-, H_2O_2, xanthine oxidase and lipid peroxidation in cerebrospinal fluid of asphyxiated cases as compared to controls.

Oxidative stress occurs if balance between cellular antioxidant defenses (SOD, catalase) is disturbed. On the basis of present study it is difficult to say whether free radicals mediated injury might have occurred during asphyxia or reventilation. Thiringer K et al[18] and McCord JM[19] studies have demonstrated that oxygen free radicals are generated during reperfusion/reventilation stage and free radical scavangers and calcium antagonists have beneficial effect.

According to Kumari SS et al[20] SOD and catalase is indirect evidence of free radical mediated injury. More subtle cooperation involves protection of SOD by catalase and peroxidase against inactivation by H_2O_2.

Significant decrease in the activity of serum catalase in encephalopathy patients could be due to less availability of NADPH. In addition reactive oxygen species generated by activated PMNs as well as the biological action of cytokines released in encephalopathy might lead to overload of superoxide radical. This over production of superoxide radical was proposed to inhibit catalase enzyme.

In the present study substantial decrease in serum vitamin E levels in HIE groups of patients as compared to control subjects ($p<0.001$) were found, Which was similar to the studied by Thorat V.N et al.[4] in 2004, depletion in the levels of serum vitamin E in HIE patients was significantly high ($P<0.001$). This confirms that depleted antioxidant status predisposing to oxidative state like encephalopathy. This also can be explained on the basis that vitamin E functions to prevent the free radical mediated lipid peroxidation.

It was also observed in present study that serum lipid peroxide level (MDA) and Catalase were significantly elevated in all three stages (I, II, and III stage) of HIE patients ($P<0.001$). Similarly SOD was also elevated in all three stages (I, II, and III stage) but p value was not significant. Vit E was significant decreased in all three stages ($p<0.001$). These findings are similar to studies done by Singh SK etall[9] and Thorate VN etall[4].

V. CONCLUSION

This present study concludes that oxinants and antioxidants status was significantly altered in neonates with Hypoxic ischemic encephalopathy (HIE). Malonyldialdehyde (MDA) in study and control group was found significant increased in neonates with HIE than healthy neonates. Among antioxidants, Catalase and SOD were found significantly increased whereas Vit E was found significantly decreased in neonates with HIE than controls.

CONFLICT OF INTEREST

None declared till now.

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