RESEARCH PAPER

Effect of High-dose Phenobarbital on Oxidative Stress in Perinatal Asphyxia: *An Open Label Randomized Controlled Trial*

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Objective: To evaluate the effect of high dose phenobarbital on lipid peroxidation and antioxidant enzymes in perinatal asphyxia.

Design: Open label, Randomized controlled trial.

Setting: Neonatal intensive care unit of a tertiary care teaching hospital.

Participants: 72 full term inborn neonates with severe birth asphyxia.

Methods: Neonates were randomized to Study (phenobarbital) group and Control group. The infants in the study group received phenobarbital infusion (40mg/kg) within first two hours of life while babies in the control group did not receive any phenobarbital. Rest of the management in both the groups was as per the unit protocol for the management of hypoxic ischemic encephalopathy. A cerebrospinal fluid examination was done at 12 ± 2 hours of life to determine the levels of superoxide dismutase, glutathione peroxidise and malonyldialdehyde. 60 neonates were followed up at 1 month of age when a detailed neurological examination was done.

Results: Four neonates in the study group and six neonates in the control group died during the study. Two neonates in the study group were lost to follow up. The cerebrospinal fluid lipid peroxides and antioxidant enzymes were significantly lower in the phenobarbital group as compared to the control group. The neurological outcome at one month follow up was found to be comparable between the two groups.

Conclusion: Phenobarbital (40mg/kg) given in the first two hours of life in term neonates with perinatal asphyxia led to a decrease in CSF levels of lipid peroxides and antioxidant enzymes at 12 ± 2 hours of life.

Key words: *Antioxidants, Management, Perinatal asphyxia, Phenobarbital*.

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Neuronal injury and the subsequent neuronal death during hypoxic ischemic encephalopathy (HIE) occurs by two basic mechanisms *viz*, rapid cell death and delayed cell death. The former occurs within minutes, is caused by glutamate receptor activation leading to increased sodium entry followed by a passive influx of chloride ions down its electro-chemical gradient along with water, causing cell swelling and lysis. The delayed cell death occurs over hours to even days and is caused by activation of N-methyl-d-aspartate (NMDA) receptors leading to entry of calcium intracellularly and the subsequent activation of several degrading enzymes such as phospholipases, nucleases, proteases etc, causing cell injury and death [1-5]. Institution of therapies post asphyxia (during the critical first six hours) have been found to be neuroprotective.

Phenobarbital with its established safety profile and low cost may hold promise as a neuroprotective agent. Its major mechanism of action is its free radical scavenging action, suppression of cerebral oxidative metabolism and the blunting of cerebral

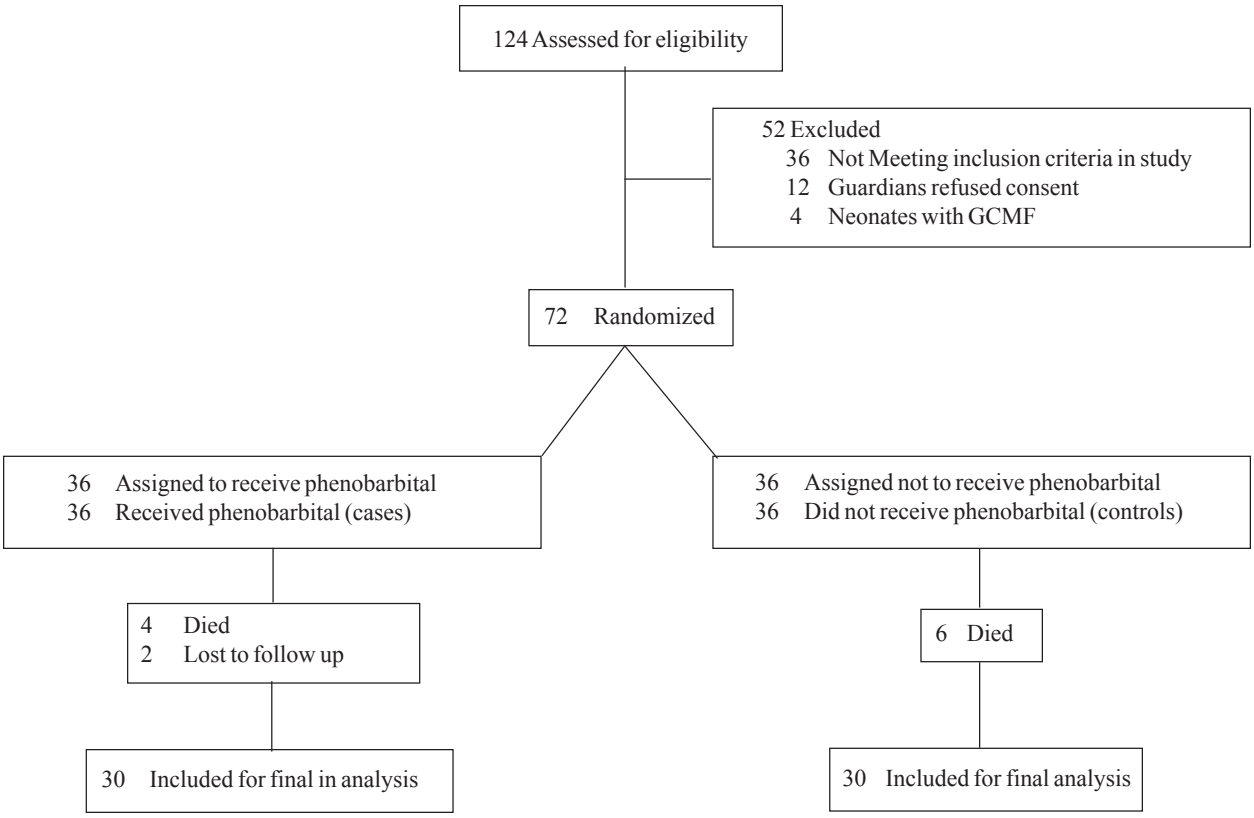

FIG.1 *Study flow diagram.*

excitotoxicity by depressing glutamate responses within the brain [6-8]. The present study was planned to evaluate the effect of phenobarbital on lipid peroxidation and antioxidant enzymes in term neonates with perinatal asphyxia.

METHODS

The study was conducted from 1 May, 2006 to 30th October, 2007 in the Neonatal Services Division, Department of Pediatrics and Biochemistry of a tertiary care teaching institution. Full term inborn babies with severe birth asphyxia who met the selection criteria (umbilical vein cord blood pH<7 and APGAR score <6 at 5 minutes) were randomized to the Study (n=36) and the Control group (n=36) using a random number table. Random number sequences were placed in opaque sealed envelopes which were opened once the baby had been resuscitated and met the selection criteria. The babies in the study group received Phenobarbital (40mg/kg) as an intravenous infusion over 60 minutes within the first 2 hours of life under continuous monitoring for heart rate, oxygen saturation, respiration and mean arterial pressure. There was no blinding and the control group received no placebo. Rest of the management in both the groups was as per the unit protocol for the management of HIE. An informed consent was obtained from the parents of all the neonates and the study was cleared by the hospital ethics committee.

Under all aseptic precautions, a cerebrospinal fluid (CSF) examination was done at 12 ± 2 hours of life in all the babies to determine the levels of lipd peroxides (malonyldialdehyde, MDA) and antioxidant enzymes, (superoxide dismutase and glutathione peroxidise [SOD, GPx]) [9-11] and CSF cell count. Protein and glucose estimation was also done to rule out meningitis. The staging of HIE was done according to the criteria of Sarnat and Sarnat [12]. Cranial ultrasound was done for all babies on day 3 and day 7 of life. Details of neonatal seizures were recorded and a detailed neurological exami-

nation was done at the time of discharge. Follow-up was done at 1 month of age when a detailed neurological examination was done and a MRI brain and an EEG were obtained.

The statistical tests used for the analysis were the unpaired student's '*t*' test and the chi-square test.

RESULTS

The baseline data including gestational age, birth weight, APGAR score and cord pH were comparable between the two groups (***Table* I**). The phenobarbital infusion was well tolerated and the temperature, heart rate, mean arterial pressure (MAP) and oxygen saturation during the infusion were within the normal limits. Six babies in the phenobarbital group and 11 in the control group received oxygen (target SPO_2 90-95%).Three babies in the phenobarbital group and four in the control group were ventilated. These data were comparable between the two groups. Four babies in the phenobarbital group and six babies in the control group died during the study. Two babies in the phenobarbital group were lost to follow up.

The mean CSF MDA level and the mean CSF SOD and GPx at 12 ± 2 hours of life was significantly lower at in the Phenobarbital group as compared to the Control group ($P<0.001$) **(*Table* II)**.

TABLE I COMPARISON OF BASELINE DATA BETWEEN THE STUDY AND THE CONTROL GROUP

Parameters	Study Group (n=30) (Mean ± SD)	Control Group (n=30) (Mean ± SD)
Birthweight (kg)	3.00 ± 0.17	2.91 ± 0.15
Gestational age (wks)	38.35 ± 1.40	39.35 ± 1.22
APGAR score at 1 min	2.1 ± 0.75	1.93 ± 0.78
At five minutes	4.7 ± 0.53	4.27 ± 0.74
pH (at birth)*	6.90 ± 1.06	6.88 ± 0.09
HIE I	7	4
II	17	15
III	6	11
Neonatal seizures†#	52 (24-120) h	78 (24-160) h

** umbilical vein cord blood pH; #Median time to become passive (range); † P <0.05; HIE: hypoxic ischemic encephalopathy.*

Seizures were controlled and became passive at day three (median 52 hours, range 24-120 hours)in the Phenobarbital group compared to day four (median 78 hours, range 24-160 hours) in the Control group ($P<0.05$). The neurological outcome at one month assessed on neurological examination, MRI brain and EEG was similar in the two groups.

DISCUSSION

The mean CSF lipid peroxide (MDA) and antioxidant levels (SOD, GPx) were found to be significantly lower in the phenobarbital group as compared to the control group. Phenobarbital infusion at 40 mg/Kg was well tolerated by all neonates. Singh, *et al*. [13], in a recent study, administered phenobarbital in a dose of 20 mg/kg within first six hours of life to near term neonates (>34 weeks) post asphyxia and reported similar findings [13].

MDA is produced as a result of lipid peroxidation and lower values of MDA imply a reduction in free radical production, possibly by phenobarbital. The significantly higher levels of antioxidant enzymes (SOD, GPx) in the Control group as compared with Phenobarbital group was possibly due to a compensatory increase in response to the higher levels of lipid peroxidation and free radical damage in the control group.

The incidence of neonatal seizures in the Phenobarbital group was comparable to that in the control group. However, the mean duration of seizures was significantly lower in the Phenobarbital group as compared to the Control Group. The

TABLE II OXYGEN FREE RADICAL AND ANTIOXIDANT ENZYMES LEVELS IN CSF AT 12 ± 2 HOURS OF LIFE

Parameters	Study Group (n=30)	Control Group (n=30)
MDA (nmol/mg protein)	1.07 ± 0.14	1.33 ± 0.10
SOD (Eu/mg protein)	4.34 ± 0.93	6.98 ± 1.19
GPx (µmoles of NADPH oxidized/min/mg protein)	5.36 ± 0.92	7.31 ± 1.59

P value <0.001; all values in mean ± SD; MOD: Malonyldialdehyde; SOD: Superoxide dismutase; GPx: Glutathione Peroxidase.

WHAT IS ALREADY KNOWN?

- Lipid peroxidation and oxygen free radical injury is involved in neuronal injury in perinatal asphyxia and HIE.

WHAT THIS STUDY ADDS?

- Phenobarbital in a dose of 40mg/kg administered in the first two hours of life decreased CSF lipid peroxide and antioxidant enzyme levels at 12±2 hours of life in neonates with perinatal asphyxia.

neurological outcome at one month of age of neonates in the Phenobarbital group was; however, not different from the Control group.

Singh, *et al*. [14] in their study on 45 term and near term infants (>34 weeks gestation) post asphyxia administered phenobarbital (20mg/kg) within 6 hours of life to 25 neonates (20 controls). The study showed a significant decrease in the incidence of seizures in the phenobarbital group (8%) as compared to the controls (40%). However, it did not alter the mortality or neurological outcome at discharge [14].

Hall, *et al*. [15] in their study on 40 term newborn infants with severe birth asphyxia administered phenobarbital (40mg/kg) within first six hours post asphyxia and showed a 27% reduction in the incidence of seizures in the phenobarbital group as compared to control group, although the difference was statistically not significant. The incidence of seizures in the present study was similar, but the time taken for seizures to become passive was significantly lesser in the study group. In their study, the neurological outcome at 3 years of age was normal in 73.3% in the Phenobarbital group (*n*=15) compared to only 18.7% in the control group(15). We found comparable neurological outcome in the two groups. However, as the follow up period in this study was only one month, a longer follow up possibly would have better elicited the differences on neurological examination. Svenningsen, *et al*. [16] had previously reported a significantly better neurological outcome at 1½ years of age in their study on full term babies with severe birth asphyxia who received phenobarbital (20mg/kg) within first 24 hours of life.

Recently, Evans, *et al.* [17] reviewed the efficacy of phenobarbital in term infants following perinatal asphyxia on death or subsequent severe neurodevelopmental disability and or the prevention of seizures. They analyzed all randomised or quasi randomised controlled trials which reported data comparing mortality, neurodevelopmental disability, neonatal seizures and adverse events, following phenobarbital in term infants compared to controls (with or without placebo) following perinatal asphyxia and concluded that barbiturates when compared to conventional therapy following perinatal asphyxia demonstrated no difference in risks of death, severe neurodevelopmental disability, or the combined outcome of death or severe neurodevelopmental disability [17].

The results from the present study showed that phenobarbital (40mg/kg) given to full term babies with severe birth asphyxia within the first 2 hours of life was safe and well tolerated. It led to a statistically significant reduction in CSF lipid peroxidation (MDA) and subsequent free radical injury and antioxidant enzyme (SOD, GPx) levels but was not associated with any significant improvement in the neurological outcome assessed at one month follow up. This could form a strong basis for conducting a larger study with a longer follow up to better document the neuroprotective role of phenobarbital in perinatal asphyxia.

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