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Biochemical marker as predictor of outcome in perinatal asphyxia.

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Abstract

Birth asphyxia is a leading cause of neonatal mortality in India. At present there is no reliable early marker to predict the outcome in these cases. We conducted this study to evaluate 8-hydroxy 2-deoxyguanosine (8-OHdG) as a biochemical marker for predicting outcome in perinatal asphyxia. Term neonates fulfilling the inclusion criteria were recruited in the study. Cord blood was collected immediately after birth. The concentration of 8-OHdG was analyzed using ELISA. These children were followed up till discharge from hospital with close monitoring for adverse events and morbidities. Neurodevelopmental assessment was done using Baroda developmental screening test up to the age of 6 months. The mean value of 8-OHdG was found to increase with severity of encephalopathy. 8-OHdG values were significantly higher among the cases that expired compared to those who survived. Developmental delay occurred in 29.4% cases. The 8-OHdG levels were significantly higher among the cases who developed developmental delay than those with normal development. It is concluded that, 8-OHdG is a good early serum biochemical marker in predicting the outcome in perinatal asphyxia

Keywords: Perinatal asphyxia, Biochemical marker, 8-hydroxy2-deoxyguanosine, Neurodevelopmental outcome

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Introduction

Perinatal asphyxia refers to an impairment of the normal oxygenation during parturition and the ensuing adverse effects on the fetus/neonate. In India, between 250,000 to 350,000 infants die each year due to perinatal asphyxia, mostly within the first three days of life. In addition, antepartum and intra-partum asphyxia contributes to as many as 300,000 to 400,000 stillbirths [1]. The outcomes of HIE are devastating and permanent making it a major burden for the patient, family and society. It is critical to identify and develop therapeutic strategies to reduce brain injury in newborns with asphyxia.

In earlier studies, several biochemical markers like lactate/creatinine ratio, neuron-specific enolase [NSE], acidic protein, uric acid brain-specific creatine kinase were investigated in the blood or cerebrospinal fluid of infants with perinatal asphyxia [2,3]. Urinary uric acid/ creatinine ratio and NSE are well established biomarkers in predicting the severity and outcome of perinatal asphyxia [3,4]. Recently studies have focussed on inflammatory cytokines like interleukin-1-beta, interleukin-6, interleukin-8. These inflammatory cytokines are implicated in the biochemical pathways leading to hypoxic-ischemic injury [5]. Other tests used for predicting prognosis are cranial tomography, magnetic resonance tomography and somatosensory evoked potentials, but these are not reliable in the first 24 hours after birth [6, 7].

Our objectives were to investigate the levels of 8-hydroxy 2-deoxyguanosine (8-ohdg) in the cord blood and to determine whether the levels can predict the outcome in perinatal asphyxia.

Material and Methods

After institute ethical committee approval, term neonates with umbilical cord blood ph $\leq$7 and Base deficit $\geq$ 16meq, APGAR $\leq$5 at 10 min and any one of the following viz: evidence of encephalopathy, evidence of fetal distress, assisted ventilation for at least 10 min after birth, evidence of any organ dysfunction, history of acute perinatal event were included as cases. Babies with major congenital abnormalities and extramural babies were excluded from the study. All babies were managed as per standard guidelines. Babies were enrolled after written informed consent from the parent.

Two ml of cord blood was collected immediately after birth and serum was separated after centrifugation. Serum

was stored at -80°C for analysis of 8-ohdg using ELISA. These children were followed up till discharge from hospital, with close monitoring for adverse events and morbidities. Neurodevelopmentaal assessment was done using Baroda developmental screening test up to 6 months of age. The categorical data was presented as frequencies and percentages. Appropriate parametric (independent students t test) or non parametric test (Mann whitney U) was used for comparing the continuous data between the two groups. All statistical analysis was carried out at 5% level of significance and p value < 0.05 was considered significant. Analysis was done using SPSS (Version 19) software

Results

Forty term neonates ranging in gestation from 37 to 42 weeks, were included in the study. Details of enrolment and follow-up of infants is given in Fig 1. Perinatal data including mode of delivery, birth weight and gestation are given in Table 1. The mean cord blood 8-ohdg level was 858.4 ±162.7. The mean value of 8-ohdg was found to increase with worsening stages of encephalopathy, with severe encephalopathy (1044.1±119.4 pg/ml) having a significantly higher value as compared to moderate (771.8±90.2 pg/ml) (p<0.00) (Table 2).

Neonates were followed up till six months and neurodevelopmental outcome was assessed using Baroda developmental screening test. Thirty one cases were evaluated for developmental outcome at six months, as there were seven deaths and two lost for follow up. 8-ohdg values were significantly higher among the cases that expired as compared to those who survived. Developmental delay occurred in 9 cases (29.03%). The 8-ohdg levels were significantly higher among the cases those had a developmental delay than cases with normal development. The 8-ohdg levels had significant negative correlation (Fig 2) with neurodevelopmental outcome at six months of age (r= - 0.765 and p<0.00).

Table 1. *Neonatal characteristics of cases*

Characteristics	**Perinatal asphyxia** (n=40)
Gestation (days)(mean±SD)	286±10.32
Mode of delivery - n (%)	
Spontaneous vaginal	22(55.0)
Instrumental	07(17.5)
Caesarean section	11(27.5)
Birth weight (kg) (mean±SD)	2.84±0.41
pH (cord blood) (mean±SD)	6.86±0.10

Table 2. *8-OHdG levels in perinatal asphyxia and outcome.*

	No (%)	**8-OHdG level (**pg/ml)	**p Value**
Encephalopathy			
Moderate	28(70)	771.8±90.2	
Severe	12(30)	1044.1±119.4	<0.001
Developmental delay[a]	9 (29.03)	1011.2±231.3	
Yes	22(70.97)	811.56±146.1	<0.001
No		5	
Survived	33(82.5)	841.3±151.6	
Expired	07(17.5)	1104.2±232.1	<0.001

[a]*n=31*

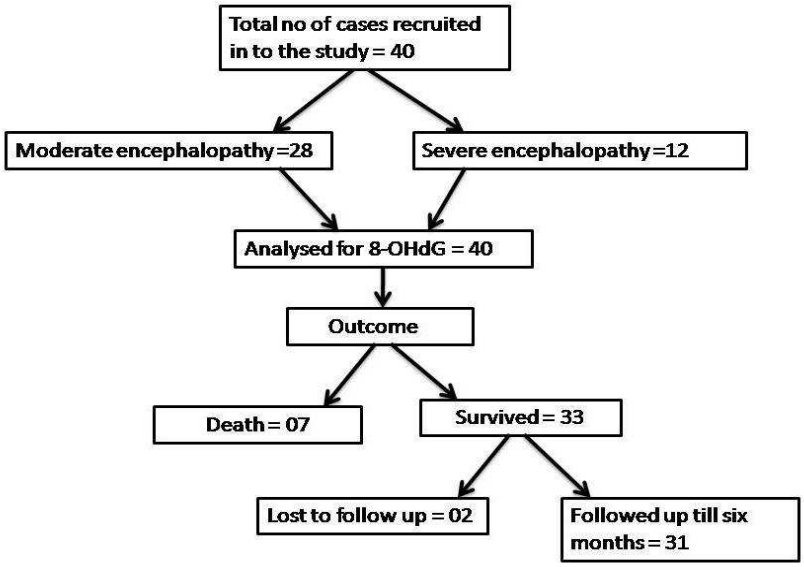

Figure 1. *Flow diagram of case enrolment and follow-up*

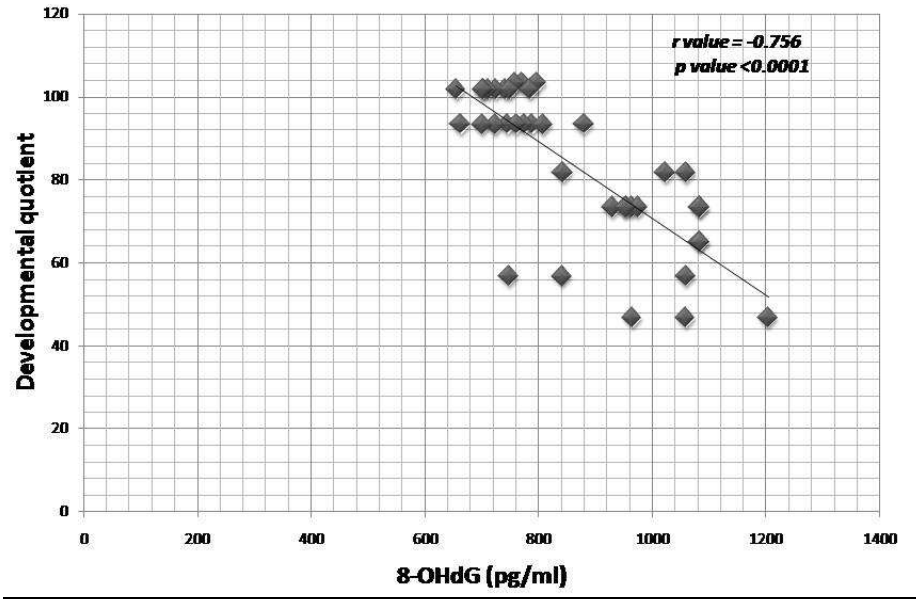

Figure 2. *Correlation between 8-OHdG levels and neurodevelpmental outcome at six months*

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Discussion

Neonatal encephalopathy is the commonest cause of mortality and neurodevelopmental disability in India[1]. Despite the newer therapeutic measures, moderate to severely affected newborns continue to have 30%-70% risk of death or disability[8-10]. In recent years, new diagnostic technologies were developed to assess brain development and to identify early brain injury. One of the important factor in improving outcome is the identification of early biomarker of brain injury that can be used to direct interventions, and provide prognostic information for parental counselling. The availability of new serum markers of risk for perinatal brain damage will allow the development of rational strategies for prevention of cerebral insults in neonates and more accurate counselling of parents[6]. Biochemical markers are also important noninvasive assessment tools for the selection of infants with moderate to severe HIE for future trials of neuroprotective therapies [11-13].

We focused on early serum biochemical marker which can be measured in cord blood for predicting the outcome. As oxidative stress plays a major role in the pathophysiology of the hypoxic-ischemic damage to the tissues, oxidative stress markers are emerging as potential prognostic tools. 8-OHdG is an important marker of oxidative stress. 8-OHdG is one of the oxidative DNA damage byproduct formed during the repair of damaged DNA in vivo by exonucleases [14-16]. The levels of 8-OHdG were found to be higher among expired cases and those with neurodevelopmental dealay at six months. 8-OHdG has not been studied till now as a marker of outcome in perinatal asphyxia. In this study,

we defined the level of this biochemical marker to predict the degree of encephalopathy and long-term outcome in term infants with perinatal asphyxia, which is of great clinical relevance. Several biochemical markers like neuron-specific enolase, protein-S glial fibrillary acidic protein, hypoxanthine, interleukin-6 and interleukin-8 have been studied among infants with perinatal asphyxia in the blood or cerebrospinal fluid [17-19]. All these have low specificity.

This study did not include a control group of healthy, nonencephalopathic infants. However, because the primary goal was to evaluate whether these biomarkers could identify infants with significant brain injury among at-risk infants, including a healthy control group was not necessary to realise our objectives. Serum levels in our study group were higher than normative values reported from cord blood of healthy newborns in a previous study [20]. The results of our study demonstrated that level of serum 8-OHdG is a useful biochemical marker for predicting the outcome in perinatal asphyxia.

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