PREDICTION OF HIE BY NUCLEATED RBC'S IN CORD BLOOD, SERUM CREATINE KINASE AND ASSESSMENT OF OUTCOME BY FOLLOW UP UPTO 6 MONTHS

Shivaprakash. N. C, Gaurav Nigam.

1. Professor, Department of Pediatrics, Adichunchanagiri Institute of Medical Sciences.
2. Final year Post-Graduate Resident, Department of Pediatrics, Adichunchanagiri Institute of Medical Sciences.

CORRESPONDING AUTHOR:
Dr. Shivaprakash. V.C,
1273, 1st Cross, MRHB Colony,
Magadi Road, Bangalore-77
E-mail: dr_sprakash@hotmail.com

ABSTRACT: OBJECTIVES: Birth asphyxia is the most common cause of preventable cerebral injury occurring in the neonatal period and contributes significantly to neonatal morbidity and mortality. This study was conducted to predict the occurrence of HIE by nucleated Red blood cells and creatinine kinase in cord blood of asphyxiated babies and assessment of outcome by follow up upto 6 months. **MATERIALS AND METHODS**: Study was conducted on 50 neonates comprising the cases and 50 neonates comprising the controls born in Adichunchanagiri Institute of Medical Sciences and Research Centre, B.G. Nagara from January 2011 to June 2012.. The cord blood samples for CK and nucleated RBCs was drawn at the time of birth and sent for analysis. Anthropometry was done at Birth ,1st month,3rd month and 6th month(final visit) , developmental assessment using DASII along with anthropometric measurements was done. **RESULTS:** The cut-off CK value of 450 U/ L has 91.3% sensitivity with a specificity of 96.3% & has a positive predictive value of 95.49 with a negative predictive value of 92.85 .The cut-off Nucleated RBCs value of >6 has 93.48% sensitivity with a specificity of 96.3% & has a positive predictive value of 97.7 % with a negative predictive value of 94.64%. NRBCs have more diagnostic value than CK with more Area Under ROC value when compared (0.989 vs. 0.986). On follow up, in the final visit, 8 cases had motor delay and 7 cases had mental delay against no developmental delay in controls. **CONCLUSION**: Prediction of HIE in the asphyxiated cases can be done using the cord blood NRBCs and Creatine kinase, & suitable interventions and intensive monitoring can be planned thereby helping in identifying the high risk cases.

INTRODUCTION: Birth asphyxia is the most important and common cause of preventable cerebral injury occurring in the neonatal period[1]. Asphyxia is the simultaneous combination of both hypoxia and hypoperfusion, which impairs tissue gas exchange leading to tissue acidosis.

In developing countries intrapartum hypoxic-ischemic injury appears to be more common. Countries reporting high incidences include Kuwait (9.4 per 1000), Malaysia (18.7 per 1000), Nigeria(26.5 per 1000), India (New Delhi 59 per 1000) and Tanzania (229 per 1000).
Globally, hypoxia of the newborn or the fetus is estimated to account for 23% of the 4 million neonatal deaths and 26% of the 3.2 million stillbirths each year.

Neonatal HIE is a progressive and evolving process, and multiple biochemical cascades contribute to its pathogenesis. The brain injury begins with the initial hypoxic-ischaemic event.

After resuscitation from the initial insult, there is a latent stage characterized by restoration of the cerebral oxidative metabolism. However, 6 to 24 hours later, there may be a further deterioration resulting in a secondary phase of energy failure the severity of which closely correlates with survival and neuro developmental sequelae.

There is a need to triage these infants on the basis of severity of hypoxia as it will determine the stage of hypoxic ischemic encephalopathy and other consequences. A variety of indicators are there to identify perinatal hypoxia including low APGAR scores, cord pH, computed tomography and MRI scans, MRI spectroscopy etc[2].

The neurological follow up of such newborn holds importance. In the form of early sequale is the development of HIE and later the neurological delay. Therefore a need for follow up of high risk neonates is a must so early intervention can be adopted to improve the outcome in these newborns.

AIMS AND OBJECTIVES OF THE STUDY:

1. To predict the occurrence of HIE by nucleated RBCs and Creatine Kinase in cord blood.
2. To assess the neurological outcome in these infants by follow up of upto 6 months.

MATERIALS AND METHODS: The study was a prospective study conducted on asphyxiated and non-asphyxiated term neonates recruited from Neonatal Intensive Care Unit (NICU) and Post natal wards of Sri Adichunchanagiri Institute of Medical Sciences, B.G. Nagara from January 2011 to June 2012. Cases and Controls comprised of asphyxiated and non-asphyxiated neonates, respectively. The blood samples from the 50 neonates comprising the cases and 50 neonates comprising the controls constituted the material for the study.

METHOD OF COLLECTION OF DATA

THE CASE GROUP included 50 neonates fulfilling the following criteria:

INCLUSION CRITERIA: (Gestational age ≥ 37 weeks, Patients whose parents are willing to take part in the study, Neonates who have experienced perinatal asphyxia)

EXCLUSION CRITERIA: (Congenital malformations, Maternal drug addiction, Neonates born to mothers who would have received magnesium sulphate within 4 hours prior to delivery or opiods, Congenital or acquired infections, Hemolytic disease of the newborn).

THE CONTROL GROUP: included 50 term apparently healthy neonates without signs of perinatal asphyxia as evidenced by normal fetal heart rate patterns, clear liquor and one minute Apgar score ≥7.

All neonates included in the study had the following done:

1) Detailed maternal history, assessment of intrauterine fetal well being by continuous electronic fetal monitoring, meconium staining of amniotic fluid, birth events, Apgar score, sex and weight of the baby were recorded. Gestational age assessed by New Ballard scoring system.
2) Cord blood sample was taken & assessed for Creatine Kinase and Nucleated RBC.

3) Thorough clinical and neurological examination was done for all the neonates included in the study. The asphyxiated neonates (case group) were monitored for seizures, hypotonia and HIE in the immediate neonatal period in the NICU. A clinical grading system by Sarnat and Sarnat was used to grade the severity of HIE. The cases were also observed for other systemic effects of asphyxia.Values for CK and NRBCs in the cord blood in both groups were compared statistically.
4) At birth anthropometric measurements were taken and these groups were followed for upto 6 months. At the second, third and fourth visit that is 1st month, 3rd month and 6th month respectively anthropometry and developmental assessment using developmental assessment scale for Indian infants (DASII) was done.

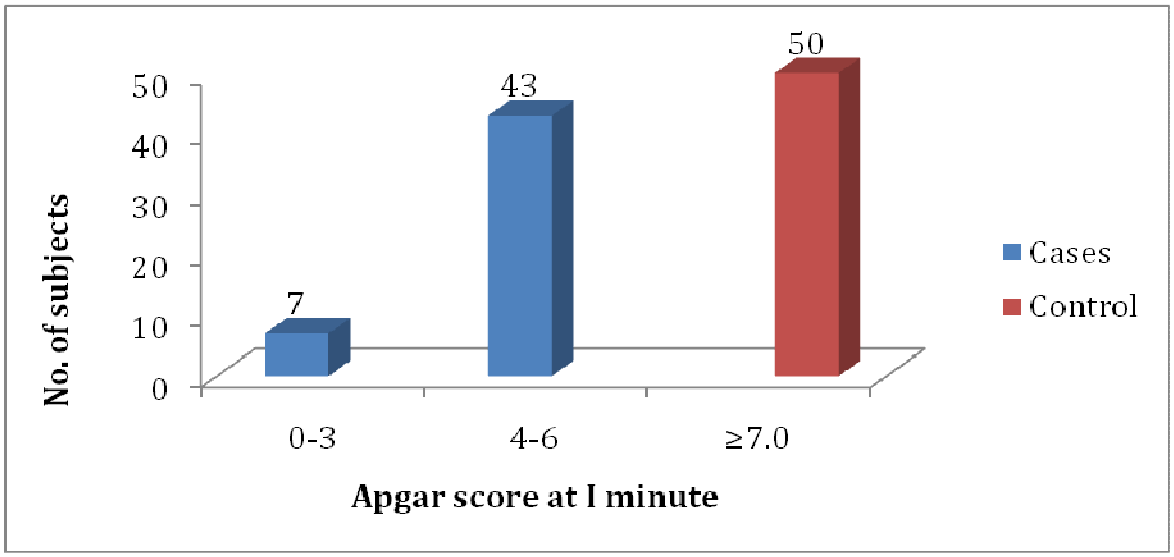
SAMPLE COLLECTION: 2 ml of blood was collected from the cord at the time of delivery of the baby for the measurement of creatine kinase and nucleated RBCs

Data collected in this study were **analysed** in SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS AND OBSERVATIONS: The cord blood samples from 50 neonates comprising the cases and 50 neonates comprising the controls constituted the material for the study.
Cord Blood for total CK and Nucleated RBC's(NRBCs`/100WBCs) was collected at birth in both cases and controls.

There is higher incidence of instrumental delivery in cases compared to controls but no significant difference in LSCS.

1(2%) had Reassuring NST and 44 (88%) had Non Reassuring NST suggestive of fetal distress in the case group. In 5 cases NST was not available. Among controls all had Reassuring NST. 46 (92%) had Thick MSAF and in 4 (8%) the amniotic fluid was clear in the case group compared to ontrols where all had clear liquor



All the 50 (100%) neonates in the case group had an Apgar score of <7 at 1 min. the difference between cases and controls Apgar score at 1 minute was significant. Among cases 28% had Apgar score between 4-6 and 72% had either greater than or equal to 7 at 5 minutes. Whereas in controls all had more than 7 at 5 minutes.

25(50%) had normal neurological examination with normal tone. 25 (50%) had decreased tone-hypotonia.

All the 50 (100%) neonates in control group had normal neurological examination.

4(8%) had no clinical evidence of HIE in the case group. 21 (42%) had Stage 1 HIE, 19(38%) had Stage 2 HIE and 6 (12%) had Stage 3 HIE during the course in NICU.In total 92% of cases developed HIE.

The mean value of NRBC's/100 WBC Count was 15.74 with standard deviation of 7.89 in cases in comparison with 1.55 with standard deviation of 0.78 in controls. This result is statistically significant.

The mean value of Creatine kinase was 679.67 with standard deviation of 169.20 in cases in comparison with 209.08 with standard deviation of 54.49 in controls. This result is statistically significant.

HIE stage	NRBC		CK	
	Mean	SD	Mean	SD
No signs of HIE	7.25	5.32	488.26	58.72
Stage 1	10.43	3.68	544.85	66.45
Stage 2	18.63	3.22	783.42	84.48
Stage 3	30.83	4.49	950.59	22.90
Inference	P=0.001		P=0.001	

Among the cases also there is a relationship between different stages of HIE and the level of CK and NRBC's with statistical significance.

At CK> 450 U/L in the cord blood has 91.3 % sensitivity and 96.3 % specificity with positive predictive value of 95.49% and negative predictive value of 92.85% for predicting the occurrence of HIE.

At NRBCs >6 NRBCs/100 WBCs in cord blood has 93.48% sensitivity with a specificity of 98.15%. LDH has a positive predictive value of 97.7% with a negative predictive value of 94.64% for predicting the occurrence of HIE.

The final outcome in the cases at 4th visit was the mean motor developmental quotient of 79.43 with standard deviation of 9.85 compared to controls who had mean motor developmental quotient of 88.00 with standard deviation of 4.50.Its is significant with p value of 0.001.

The final outcome in the cases at 4th visit was the mean mental developmental quotient of 81.68 with standard deviation of 9.03 compared to mean mental developmental quotient of 90.15 with standard deviation of 3.94 in controls. It is significant with p value of 0.001.

DISCUSSION: Birth asphyxia is a common complication that occurs between 2-10%of deliveries[1] and is an important cause of preventable cerebral injury occurring in the neonatal period, but although asphyxia at birth is a commonly made diagnosis, there is generally no accepted definition

for it. In India, 8.4% of inborn babies have a 1 minute APGAR score less than 7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE)[2]. The APGAR score has a limited role in predicting HIE and the long-term squeal. Several studies have shown that cerebral function monitoring using non invasive techniques, such as EEG within six hours of birth, cranial ultrasonography, Doppler measurements of cerebral blood flow, MRI and estimation of neurophysiological markers such as CK-BB, brain specific LDH isomer, glutamate and neuron specific enolase in CSF are all useful in predicting both the immediate dysfunction and the long term outcome[3,4]. However these facilities are not readily available in our country. The signs of asphyxial injury are nonspecific and overlap with other illnesses. In the absence of perinatal records, it is difficult to retrospectively diagnose perinatal asphyxia. Thus if any predictor with high sensitivity and specificity could be found out to predict HIE then active therapeutic intervention can be offered and can be used for prognostication as well.

In the present study an attempt has been made to ascertain whether CK and NRBCs can distinguish between asphyxiated and non asphyxiated babies and predict occurrence of HIE and also to assess the outcome of these babies. This study also tried to establish the prognosis of different stages of HIE especially in the rural setup. These tests are routinely available and thus can be very helpful.

Comparative study of baseline characteristics of cases and controls compared between study by H.Boskabadi et al[1] and present study.

Characteristics		H.Boskabadi et al		Present study	
		cases	controls	Cases	controls
Number		42	49	50	50
Birth weigh (grams)	Mean	2920	3070	2870	2910
	Standard deviation	405	416	440	380
Mode of delivery	normal	31%	71%	48%	68%
	instrumental			20%	-
	cesarean	69%	29%	32%	32%
Sex	Male	26	25	25	28
	Female	16	24	25	22
APGAR 1’	Mean	4.0	8.4	4.92	7.18
	SD	1.6	.7	1.21	.39
APGAR 5’	Mean	5.6	8.8	7.18	9
	SD	1.8	.6	1.08	0
NRBC’s/100 WBCs	Mean	18.63	3.87	15.74	1.55
	SD	16.63	5.06	7.89	.78

In our study at the time of admission 25 cases were having normal neurological examination and 25 were having hypotonia(mild/moderate/severe) and 23 cases had seizures where as all the 50 controls had normal neurological examination.

In our study birth asphyxia showed statistically significant association with meconium stained amniotic fluid. MSAF is one of the signs of fetal distress in-utero and can be attributed to asphyxia.

There was no significant association between birth asphyxia and parity of mothers (p> 0.05) which is comparable to Reddy S et al[5] and Khreisat WH et al[6] and H.Boskabadi et al[7].

Evidence of fetal distress in the form of non reassuring NST was seen in 88% of cases in the present study compared to 92% in Reddy S et al[5].

In our study among the cases 4(8%) showed no signs of HIE, 21(42%) had stage 1 HIE, 19(38%) were having stage 2 HIE and 6(12%) had stage 3 HIE.

In our study creatine kinase (CK) between cases and controls were having statistically significant difference. And among the cases 92% developed HIE therefore it can predict HIE with quite an accuracy.

A study by Agrawal et al[8] and D H Karunatilaka[9] had shown that creatine kinase increases in asphyxiated group and that the level of rise is proportionate to the stage of HIE.

In our study the CK value also varied with different stages of HIE. And thus it can be prognosticated at earlier level and appropriate intervention can be done.

HIE stage	**CK**	
	Mean	**SD**
No signs of HIE	488.26	58.72
Stage 1	544.85	66.45
Stage 2	783.42	84.48
Stage 3	950.59	22.90
Inference	P=0.001	

As comparable to the study by H. Boskabadi et al[7], in the present study there is significant statistical difference between the cases and the controls in terms of nucleated RBCs in the cord blood with p 0.001 and among cases 92% developed HIE therefore it can predict HIE with precision.

HIE stage	**NRBC**	
	Mean	**SD**
No signs of HIE	7.25	5.32
Stage 1	10.43	3.68
Stage 2	18.63	3.22
Stage 3	30.83	4.49
Inference	P=0.001	

Present study: sensitivity, specificity, PPV, NPV and AUROC values for NRBCs and CK for predicting HIE.

	Sensitivity	**Specificity**	**PPV**	**NPV**	**AUROC**
NRBCs(>6 NRBCs/100WBC)	93.48%	98.15%	97.7	94.64	0.989
CK(>450.5 U/L)	91.3%	96.3%	95.49%	92.85%	0.986

NEUOROLOGICAL ASSESSMENT: In the present study there is significant delay in development in cases compared to control. Delay was taken as developmental quotient below 70 and it was calculated using developmental delay scale for Indian infants(DASII).

Among the cases, 16 out of 48 neonates during second visit had motor developmental delay and 12 had mental delay. whereas in controls 1 had motor as well as mental developmental delay. In the 3rd visit 13 cases had motor developmental delay and 7 had mental developmental delay, whereas 1 control had motor developmental delay in the 3rd visit, no control had mental developmental delay in this visit.

In the final (4th) visit 8 cases had motor developmental delay and 7 had mental developmental delay. No developmental delay in any domain in controls in final visit.

There is a significant difference between development quotient between cases and control with p value <0.001 between the two domains of development namely motor and mental, Motor developmental delay is seen more in comparison to mental developmental delay.

Among the cases the trend of developmental delay in both the domains in different visits is descending in nature i.e. during second visit number of patient having motor delay was 16, in the next visit it came down to 13 and in the final visit only 8 cases had motor developmental delay.

A similar trend is observed in mental developmental delay at second visit it was 12, in the third and final visit it was 7 cases. In the control group there was only one patient who had motor delay in second and third visit and mental delay only in second visit. This patient did not follow up for the final visit and thus etiology of delay could not be found out. But asphyxia was ruled out.

HIE stage	**DmeQ(4th visit)**		**DmoQ(4th visit)**	
	Mean	**SD**	**Mean**	**SD**
No signs of HIE	93.00	0	89.75	3.77
Stage 1	86.86	5.51	84.79	5.97
Stage 2	76.72	5.78	75.26	6.23
Stage 3	65.50	1.73	59.75	7.09
Inference	P=0.001		P=0.001	

Among the cases the motor and developmental quotient also varies with the stages of HIE as assessed in the final visit. The mean DmeQ and DmoQ are 93 and 89.75 among the cases who showed no signs of HIE. The mean DmeQ and DmoQ is 86.6 and 84.79 in stage 1, 76.2 and 75.26 in stage 2 and 65.50 and 59.75 in stage 3 respectively.

Among the cases, 2 stages 3 HIE cases dropped follow up after third visit and could not be traced. Thus their ophthalmic and hearing evaluation along with developmental assessment could not be done in the final visit. Other than them no cases or control showed any obvious hearing or ophthalmic problem.

In our study in the second visit 48 cases and 50 controls attended, In third visit 49 cases and 49 controls attended and in the fourth visit 47 cases and 48 controls attended. These variations were due to various reasons such as job of the parents(daily wage workers), transportation etc. But there are various studies in the past[10] for various topics involving follow up.

Thus based on NRBCs and CK value, HIE can be predicted and intervention can be started early (Neuroprotection, monitoring etc).Also the developmental quotient in different stages of HIE also varies. Thus appropriate interventions for long term sequale can also be taken in the form of physiotherapy, stimulation, speech therapy etc.

CONCLUSION: Birth asphyxia-hypoxic ischaemic insult has been incriminated as one of the most important cause of perinatal mortality and contributes significantly to neonatal morbidity and mortality.

There is a need to identify neonates at high risk for HIE and early neonatal death as a consequence of perinatal hypoxia and the long term sequelae in the form of motor and mental retardation. Interventions can be taken up in these cases to improve the outcome. EEG, cranial ultrasonography, CT, MRI, cerebral blood flow velocities, estimation of neurophysiological markers are useful in predicting both the immediate dysfunction and the long term outcome.

Increase in the total CK values and Nucleated RBC's are seen in asphyxiated newborns and is proportional with the stage of HIE which in turn has a relation with developmental delay.

There is a significant difference between the CK values of cases and controls and also among the cases in different stages of HIE. There is also significant difference between the NRBCs/100WBCs among cases and control and also among the cases in different stages of HIE which in turn has a relation with the developmental delay as a later squeal.

Estimation of nucleated RBC's and CK in the cord blood can help distinguish an asphyxiated from a non-asphyxiated term neonate and predict occurrence of HIE.

When such neonates are followed up they showed developmental delay between cases and controls.

So the present study can be beneficial to predict the occurrence and severity of HIE and for appropriate intervention at that time and also to prognosticate the later outcome and identification of high risk neonates and follow up and intervention in them to avoid the developmental delay.

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