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Original Research Article

Study of hepatic dysfunction in full term asphyxiated neonates

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Abstract

Background: Birth asphyxia resulting in hypoxic-ischemic encephalopathy (HIE) remains a major cause of neonatal mortality and morbidity worldwide. Different organ systems of the body are affected in birth asphyxia, liver being one of them.
Method and Material: Full term neonates with APGAR score of ≤7 at five minute of life or Failure to breath spontaneously immediately after birth or Requiring resuscitative measures to sustain life after birth; Along with Umbilical cord pH <7 or base deficit ≥16mmol/lt were included. Neonates with Major congenital malformation, chromosomal abnormalities, Birth trauma and Sepsis were excluded. APGAR was recorded at 1st, and 5th minute of life. Cord blood ABG was done to diagnose perinatal asphyxia. Neonates were grouped according to Sarnat and Sarnat stages of HIE. Serums AST, ALT, ALP of asphyxiated neonates were estimated between 48 to 72 hours of life.
Results: Total 100 neonates were enrolled in the study with mean age 38±1.27 weeks and mean birth weight 2.8±0.4 kg. The M: F ratio was 1.4:1. Among 100 asphyxiated neonates 84% had HIE of varying category and 53% had hypoxic hepatic injury. Hepatic dysfunction was found in 2 of 16(12.5%) neonates with no HIE, 8 of 19(42.1%) neonates with HIE1, 28 of 45(62.2%) neonates in HIE2 and 15 of 20 (75%) neonates in HIE3. There was statistical significance in occurrence of HHI according to stages of HIE. Positive correlation was noted between serum levels of liver enzymes and HIE staging of neonates.
Conclusion: Majority of Asphyxiated neonates developed hypoxic hepatic injury. ALT, AST and ALP has positive correlation with increasing severity of HIE staging.

Keywords: Birth asphyxia, umbilical cord PH and base deficit, hypoxic ischemic encephalopathy, hepatic dysfunction

Introduction

Perinatalasphyxia is defined as insufficient blood gas exchange during the birth process resulting in hypoxia, hypercarbia and metabolic acidosis [1]. The WHO defines perinatal asphyxia as "Failure to initiate or sustain breathing at birth [2]". The frequency of perinatal asphyxia is approximately 1% to 1.5% of live births in developed countries with advanced obstetric/neonatal care, and more number of infants in resource limited countries. It occurs in 0.5% of live born newborns >36 weeks gestation and accounts for 20% perinatal deaths (50% if still births included) [3].

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BA has damaging effects on several body organ systems. Hypoxia causes damage to almost every tissue and organ. In response to hypoxic-ischemic insult to the fetus, a series of protective reflexes, called diving sea reflexes, get initiated to prevent damage to more vital organs (brain, heart, and adrenals) at the expense of lesser vital organs (kidney, lungs, gastrointestinal tract, liver, and spleen) by an attempt to redistribute available blood flow [4]. Ultimately it results in serious organ damage and multiorgan failure. Central nervous system dysfunction associated with perinatal asphyxia is referred to as Hypoxic ischemic encephalopathy (HIE). HIE is of foremost concern in an asphyxiated neonate because of its potential to cause serious long-term neuromotor sequelae among survivors.

Hepatic involvement is often found in the subjects as it is highly involved in so many metabolic processes. Liver cell injury commonly occurs after perinatal asphyxia, and is similar to shock liver syndrome [5]. It is represented as an early, abrupt, and transient (peak concentration 24-72 hours after) insult with an increase in aminotransferases [aspartate transferase (AST) and alanine transferase (ALT)] and alkaline phosphatase (ALP) plasma activity. Later on, the peak aminotransferase level returns to near normal within 10 days.

APGAR score and blood gas analysis are used for diagnosing and assessing the severity of perinatal asphyxia. In developing countries like India most of the deliveries occur peripherally away from institutions where it is difficult to get APGAR and perinatal records. Diagnosis of perinatal asphyxia is mostly established retrospectively in our country. But it is difficult to diagnose perinatal asphyxia retrospectively in the absence of perinatal records, using Apgar scores alone has limitations in predicting the immediate outcome such as development of HIE and the long-term sequelae. As APGAR scores are affected by a number of factors such as maternal drugs, neuromuscular disorders, birth trauma and prematurity, which may result in low Apgar scores in the absence of asphyxia [6].

Knowledge of the behavior of AST and ALT activity may have important implications in the diagnosis, early treatment and there by outcome of perinatal asphyxia. Therefore, this study was designed to find out any correlation existing between hepatic dysfunction and the severity of perinatal asphyxia with respect to hypoxic ischemic encephalopathy by sarnat and sarnat staging and to whether hepatic dysfunction can be used as a diagnostic tool in cases of perinatal asphyxia.

Several studies are available on BA-related injury to brain, kidneys, and heart, there are limited studies regarding BA-induced hepatic injury. Hence this study was conducted to see the extent of hepatic injury in birth asphyxia and to check whether liver enzymes can be used in diagnosing birth asphyxia.

Methodology

Source of data: All Term neonates with birth asphyxia admitted in NICU.
Type of study: Hospital based prospective cross sectional study.

Inclusion criteria

Full term neonates with one of following:

1. APGAR score of ≤7 at five minute of life (or).
2. Failure to breath spontaneously immediately after birth (or).
3. Requiring resuscitative measures to sustain life after birth Along with Umbilical cord ABG abnormalities (pH less than 7 or base deficit more than or equal to 16mmol/lt) 85.

Exclusion criteria

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1. Major congenital malformations.
2. Chromosomal anomalies.
3. Birth trauma
4. Sepsis

- Informed written consent from parents of neonates meeting inclusion and exclusion criteria was taken.
- APGAR was recorded at 1stand 5th minute of life.
- Cord blood pH and ABG were done to diagnose perinatal asphyxia.
- Sampling of cord blood for pH and ABG was done using following steps: Immediately after birth of asphyxiated newborn

1. A segment of cord was isolated by two sets of clamps.
2. Clamped cord segment was removed.
3. One mL of blood was collected in heparinized syringe from doubly clamped segment of umbilical cord for Arterial Blood Gas Analysis (ABG).

- Neonates with birth asphyxia were grouped into mild asphyxia (APGAR 6, 7), moderate asphyxia (APGAR 4-5) and severe birth asphyxia (APGAR ≤3) 87 based on Apgar score at 5 minutes.
- Demographic and clinical data including gestational age; birth weight; sex; delivery route; clinically identifiable sentinel event (cord prolapses, fetal distress, uterine rupture, abruption of placenta, difficult delivery), resuscitation information, presence of seizures, were recorded.
- All neonates were staged using the Sarnat and Sarnat staging system of HIE.
- Serum AST, ALT, ALP, of asphyxiated babies were estimated between 48 to 72 hours of life.
- The criteria for liver impairment were taken as follows: ALT >50 U/L, AST >120 U/L, ALP >420 U/L.
- Neonates who died within 48 hours of life and before estimation of serum enzymes were excluded from the study.
- Neonates with HIE were uniformly managed as per NICU protocol. Mortality and short-term outcome were assessed till discharge.
- Antibiotics were given as and when needed after obtaining blood culture.
- Sepsis was diagnosed based on clinical findings [tachycardia (heart rate >110, tachypnea, abdominal distension] along with leukopenia (counts less than 5000/mm3), CRP positive, chest x-ray suggestive of pneumonia or positive blood culture.

Results

Table 1: Comparison of BA severity with ALT, AST and ALP

BA Severity	n	ALT		AST		ALP	
		Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Mild	43	41.58	14.37	73.84	30.37	169.58	77.80
Moderate	44	61.59	22.04	98.18	34.59	242.07	107.16
Severe	13	89.23	30.38	133.23	45.82	316.62	135.22
Total	100	56.58	25.68	92.27	39.35	220.59	110.99
F-value		29.3951		15.9590		12.6354	
P-value		0.0001*		0.0001*		0.0001*	

In Mild BA, Mean ALT was 41.58 ± 14.37 IU/L, Mean AST was 73.84 ± 30.37 IU/L, Mean ALP was 169.58± 77.80 IU/L.
In Moderate BA, Mean ALT was 61.59 ±22.04 IU/L, Mean AST was 98.18 ± 34.59 IU/Land Mean ALP was 242.07 ± 107.16 IU/L.

In Severe BA, Mean ALT was 89.23 ± 30.38 IU/L, Mean AST was 133.23 ± 45.82 IU/L and Mean ALP was 316.62 ± 135.22 IU/L.
There was a significant difference (p<0.05) in Mean ALT, AST and ALP Comparison With respect to Birth Asphyxia Severity.

Table 2: Comparison of HIE with ALT, AST and ALP

HIE	n	ALT		AST		ALP	
		Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
No HIE	16	35.81	12.35	53.31	22.43	130.50	51.56
HIE 1	19	44.95	15.79	78.84	31.59	184.26	99.77
HIE 2	45	60.20	23.12	99.56	33.19	248.07	105.82
HIE 3	20	76.10	30.18	119.80	42.35	265.35	121.12
Total	100	56.58	25.68	92.27	39.35	220.59	110.99
F-value		11.8859		13.4013		7.3971	
P-value		0.0001*		0.0001*		0.0001*	

In No HIE, Mean ALT was 35.81 ± 12.25 IU/L, Mean AST was 53.31 ± 22.43 IU/L and Mean ALP was 130.50 ± 51.56 IU/L.
In HIE 1, Mean ALT was 44.95 ± 15.79 IU/L, Mean AST was 78.84 ± 31.59 IU/L and Mean ALP was 184.26 ± 99.77 IU/L.
In HIE2, Mean ALT was 60.20 ± 23.12 IU/L, Mean AST was 99.56 ± 33.19 IU/L and Mean ALP was 248.07 ± 105.82 IU/L.
In HIE 3, Mean ALT was 76.10 ± 30.18 IU/L, Mean AST was 119.80 ± 42.35 IU/L and Mean ALP was 265.35 ± 121.12 IU/L.
There was a significant difference (p<0.05) in Mean ALT, AST and ALP Comparison With respect to HIE.

Table 3: Correlation between HIE with ALT, AST and ALP

Variables	Correlation between HIE with			
	N	Spearman R	t-value	p-level
ALT	100	0.5281	6.1558	0.0001*
AST	100	0.5443	6.4234	0.0001*
ALP	100	0.4555	5.0655	0.0001*

There was positive correlation between liver enzymes with HIE grading.

Table 4: Neonates with raised ALT, AST and ALP in HIE stages

	No HIE	HIE 1	HIE 2	HIE 3	Total	$\chi 2$	p-value
ALT							
≤50	14	11	17	6	48	15.2269	0.0016*
>50	2	8	28	14	52		
Percentage	12.5%	42.1%	62.2%	70%	52%		
AST							
≤120	15	15	24	10	64	11.9124	0.0077*
>120	1	4	21	10	36		
Percentage	6.6%	21.05%	46.6%	50%	36%		
ALP							
≤420	16	17	36	14	83	8.9196	0.0304*
>420	0	2	9	6	17		
Percentage	0%	10.5%	20%	30%	17%		
Total	16	19	45	20	100		

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Raised ALT of >50 IU/l was noticed in 52 (52%) of neonates. 2 (12.5%) in NO HIE, 8(42.1%) in HIE 1, 28 (62.2%) in HIE2 and 4(70%) in HIE 3.

Raised AST of >120 was noticed in 36(36%) neonates.1 (6.6%) in NO HIE, 4 (21.05%) in HIE 1, 21 (46.6%) in HIE 2 and 10 (50%) inHIE 3.

Raised ALP of >420 was noted in 17(17%) neonates, 0(0%) in NO HIE, 2 (10.5%) with HIE 1, 9 (20%) with HIE 2 and 6 (30%) with HIE 3.

There was a significant difference ($p<0.05$) in number of neonates with raised ALT, AST and ALP with respect to HIE distribution.

Table 5: Comparison of ALT, AST and ALP levels in neonates with HHI vs. neonates without HHI

Variable	HHI	N	Mean	SD	SE	t-value	P-value
ALT	Without HHI	47	35.98	9.36	1.37	-11.5497	0.0001*
	With HHI	53	74.85	21.31	2.93		
AST	Without HHI	47	62.98	21.89	3.19	-9.8257	0.0001*
	With HHI	53	118.25	32.58	4.48		
ALP	Without HHI	47	156.81	47.69	6.96	-6.4162	0.0001*
	With HHI	53	277.15	120.43	16.54		

*$p<0.05$

In Subjects without Hypoxic Hepatic Injury, Mean ALT was 35.98 ± 9.36 IU/L, Mean AST was 62.98 ± 21.89 IU/L and Mean ALP was 156.81 ± 47.69 IU/L.

In Hypoxic Hepatic Injury, Mean ALT was 74.85 ± 21.31 IU/L, Mean AST was 118.25 ± 32.58 IU/L and Mean ALP was 277.15 ± 120.43 IU/L.

There was a significant difference in Mean ALT, AST and ALP Comparison With respect to Hypoxic Hepatic Injury.

Table 6: Comparison of outcome with ALT, AST and ALP

Variable	Outcome	n	Mean	SD	SE	t-value	P-value
ALT	Discharged	87	52.24	20.62	2.21	-4.8405	0.0001*
	Died	13	85.62	36.66	10.17		
AST	Discharged	87	86.57	35.66	3.82	-4.0204	0.0001*
	Died	13	130.38	43.08	11.95		
ALP	Discharged	87	209.68	106.07	11.37	-2.6174	0.0103*
	Died	13	293.62	119.79	33.22		

*$p<0.05$

In Discharged subjects, Mean ALT was 52.24 ± 20.62IU/L, Mean AST was 86.57±35.66IU/L and Mean ALP was 209.68 ± 106.07 IU/L.

Among whom Died, Mean ALT was 85.62 ± 36.66 IU/L, Mean AST was 130.38 ± 43.08 IU/L and Mean ALP was 293.62 ± 119.52 IU/L.

There was a significant difference in Mean ALT, AST and ALP Comparison With respect to Outcome.

Discussion

Increasing trend in serum levels of ALT, AST and ALP was noted with decreasing APGAR scores and increase in severity of birth asphyxia from moderate to severe and difference was statistically significant. Similar results were obtained by Godambe *et al*. [7]. They used ALT values of more than 40IU/L or more than twice the control group as criteria for liver injury. The mean ALT level was noted to increase from 35.3 ±28 [8]. IU/L in mild asphyxia to 65.6 ± 33 [2]. IU/L in severe asphyxia. Similar results were observed by other workers who noted a

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rise from 44 ± 61 [9]. IU/L in mild to 59.5 ± 108 IU/L in severe asphyxia. The difference in the means of the serum levels of enzymes in neonates suggest that the values of enzymes in the circulation increases with severity of PA. So, with these, the values of these enzymes may probably suggest the severity of the hypoxic-ischaemic insult that a baby has suffered.

Among the enrolled neonates (n=100), HIE developed in 84cases (84%) with 19% cases in stage I, 45% in stage II and 20% in stage III. No HIE was noted in 16% of neonates. As HIE progressed from NoHIE to HIE 3, decreasing trend of mean APGAR was noted and it was statistically significant with respect to HIE. There was a significant difference in Mean cord blood PH distribution with respect to HIE. Similar to study conducted by Gurdeep Singh Dhanjal *et al*. [8].

A study by Mukesh choudhary *et al*. [9] noted 52% had HIE with 14% in stage 1,26% in stage,12% in stage 3 and 48% had no HIE. There were less number of babies with no HIE in our study compared to Mukesh Choudhary *et al*. as they had used APGAR score a sole indicator in diagnosing birth asphyxia, we along with APGAR used umbilical cord PH and base excess as indicators for diagnosing birth asphyxia, which is more reliable than APGAR alone in diagnosing asphyxiated babies.

In our study 53(53%) neonates developed hypoxic hepatic injury, 2 out of 16(12.5%) in no HIE, 8 of 19(42.1%) in HIE1, 28 of 45(62.2%) in HIE 2, and 15 of 20 (75%) in HIE 3. Our observation of 53% of newborns with BA developing HHI lies well within the range of previous observations of 39% to 87% prevalence of this condition.

This wide range could be due to variations in criteria used in different studies to diagnose BA as well as HHI, severity of BA, time-points used for serum transaminase estimation, cut-off used for liver enzyme elevation, gestational age of the babies, and other obstetrical or neonatal factors. A previous prospective study had shown HHI in 56% of babies with BA, similar to our observation. All these observations, including our study, suggest that HHI is common in babies with HIE.

In the present study, ALT levels were elevated in 52% neonates with PA, AST levels were elevated in 38% of babies and ALP was elevated in 17% of babies. PA affects multiple organs in body by the hypoxic ischemic insult before development of encephalopathy. This organ damage results in leakage of intracellular enzymes such as ALT, AST and many other enzymes in circulation. Many of the effects of these multiple organ involvement usually go unnoticed because they are transient and often resolve completely without sequelae. One of the earliest hepatocellular changes in hypoxia is the formation of plasma membrane protrusions called blebs. These early changes are reversible. Irreversible injury occur when a plasma membrane bleb bursts, causing abrupt failure of the plasma membrane permeability barrier and release of intracellular enzymes and metabolites. There is leakage of cytoplasmic enzymes from cell, but minimal release of other enzymes. Thus necroinflammatory change in liver leads to release of AST and ALT but not of mitochondrial isoenzyme of AST or to release of ALP. This might be the cause of comparatively less number of neonates having increased serum ALP, AST than ALT.

There was an increasing trend in mean ALT from 35.81±12.35 IU/L in no HIE to 76.10± 30.18 IU/L in HIE 3.There was significant difference in levels of ALT according to stages of HIE.

There was also increasing trend in mean AST from 53.31±22.43IU/L in no HIE to 119.80±42.35 in HIE stage 3. Significant difference was found in levels of AST according to stages of HIE

Likewise mean ALP was also found to be increasing from 130.50±51.56 IU/L in no HIE to 265.35±121.12 IU/L in HIE 3and it was statistically significant. Islam *et al*. [10] and many other researchers have shown similar relationship between aminotransferases, alkaline phosphatase and HIE. The serum levels of all these enzymes increases with the occurrence of

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HIE. The mean value of AST, ALT and ALP at 48-72 hours of life were significantly higher in babies that developed HIE when compared to babies that did not develop HIE. There was a significant difference in Mean ALT, AST and ALP Comparison With respect to Hypoxic Hepatic Injury. Nanda Chhavi *et al.* [11] and Mukesh Choudhary *et al.* [9] noticed similar results in their study.

The increase in AST, ALT, ALP showed a significant positive correlation with the severity of asphyxia and the stages of HIE. Deepak Sharma [12] *et al*., and MD Tariqul [10] *et al*., also noted positive significant correlation between HIE staging and AST, ALT and ALP.

Conclusion

The hepatic injury is a useful marker for assessment of severity of asphyxia injury. we concluded that estimation of liver enzymes (ALT, AST and ALP) at 48 to 72 hours of life can be useful as diagnostic tool to differentiate asphyxiated neonates from non-asphyxiated neonates and to assess the severity and outcome of perinatal asphyxia because of easy availability and feasibility of tests and to prevent the dreaded outcomes as far as possible. The result of present study could be used in referral hospitals who receive sick neonates and where birth details are not readily available.

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