

## Original Research Article

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# Hepatic dysfunction in asphyxiated neonates: a prospective case-control study

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## ABSTRACT

**Background:** Birth asphyxia can lead to hypoxic damage to liver at cellular level which leads to release of liver enzymes in blood as well as altered liver function. This study aimed to study the alteration of hepatic function in birth asphyxia and correlate it with different stages of hypoxic ischemic encephalopathy (HIE).

**Method:** A case control study was conducted at SMIMER Surat which involved 115 cases and 115 controls. Cases were full term neonates admitted in the NICU with an APGAR score of 6 or less at 1 minute of birth while controls were those neonates with an APGAR score of 7 or more. Blood samples were taken at 72 hours of life and liver function tests of the 2 groups were compared.

**Results:** The difference in aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) levels of the cases and controls were statistically significant ( $p<0.05$ ). However, difference in total protein and total bilirubin in between the 2 groups was statistically insignificant. The study also found that the difference in AST, ALT and LDH was statistically significant between HIE stage 1 and stage 2 ( $p<0.5$ ), between HIE stage 1 and stage 3 ( $p<0.5$ ) but insignificant between HIE stage 2 and stage 3 ( $p>0.5$ ).

**Conclusions:** The present study showed that the serum levels of hepatic enzymes were higher in full term neonates with perinatal asphyxia than full term neonates without perinatal asphyxia at the age of 72 hours. The enzyme levels showed an increasing trend with increasing severity of HIE.

**Keywords:** Asphyxia, HIE, APGAR, Hepatic function

## INTRODUCTION

Perinatal hypoxia is one of the leading causes of perinatal morbidity and mortality in developing countries. Though there are more and more studies about understanding the mechanisms leading to birth asphyxia, early determination of tissue damages due to birth asphyxia are still lacking.<sup>1</sup> One of the defense mechanisms that operate when a fetus is exposed to wide spread hypoxic-ischemic injury is the ability to divert cardiac output to the brain, heart and adrenals at the expense of other organs such as the kidney, gastrointestinal tract, skin, liver, muscles and lungs called as diving reflex.<sup>2</sup> Since the liver is also the major site for synthesis of important proteins like

albumin and clotting factors, hypoxic damage to the liver can result in decreased synthesis of these proteins which can be reflected by a lower serum protein and albumin level and a prolonged prothrombin time.<sup>3</sup> Owing to the limitations of the Apgar scoring system in predicting the immediate outcome such as HIE and long-term sequelae, other methods, including the assaying for these enzymes, have been used for grading severity of perinatal asphyxia, predicting immediate neurological dysfunction and the long term outcome of HIE.<sup>4</sup> There is a dearth of such studies in developing countries where perinatal asphyxia is more common. This study was therefore carried out to assess the levels of these enzymes in full term babies

delivered at SMIMER hospital, Surat with perinatal asphyxia.

It was aimed that the results of the present study will contribute to the knowledge on surrogates for more accurately assessing the severity of perinatal asphyxia and early identification of babies that are likely to develop HIE and may subsequently need neuroprotective measures that have been shown to mitigate the adverse effect of severe perinatal asphyxia.

## METHODS

It is hospital-based case control study conducted in neonatal ICU, department of pediatrics, Surat municipal institute of medical education and research (SMIMER), Surat city. Study was conducted over period of 18 months and data analysis was conducted for 15 months.

It was calculated by using open epi software by considering data of a previous reference study conducted by Choudhary et al, a prospective case control study on hepatic dysfunction in asphyxiated neonates.<sup>5</sup> The level of significance was set at 95%. The calculated sample size was a total of 230 neonates of which 115 were included as cases and 115 were included in study group.

Cases and controls were selected randomly after applying the inclusion and exclusion criteria irrespective of body weight and gender of the neonates. Full term neonates admitted in the NICU with an APGAR score of 6 or less at 1 min of birth were taken as cases whereas full term neonates with APGAR of 7/more were taken as controls.

Neonates whose parents did not give consent for the study, neonates with major congenital malformations, neonates with suspected metabolic disease, neonates born to mothers who received general anesthesia, pethidine, phenobarbitone or any other drugs which are likely to cause neonatal depression in babies, neonates with a gestational age of <37 completed weeks and neonates transferred from another hospital were excluded from study. Data collection was done by using a structured pre-prepared case Proforma to enter the patient details, detailed clinical history including maternal antenatal history and history during labour, need for resuscitation and APGAR score at 1 min and 5 min of birth.

According to NNPD 2000, the cases were classified into two groups. Those with APGAR score of 4-6 at 1 min (moderate birth asphyxia) were in one group and those with APGAR score of 3 or less at 1 min (severe birth asphyxia) were in another group (Apgar scores are also useful for predicting long term outcome in infants with perinatal asphyxia).<sup>6-8</sup> The cases were also classified in 3 groups depending on stages of HIE using the Sarnat and Sarnat scoring system.<sup>9</sup>

The blood samples were collected at 72 hours of life which were then sent to the laboratory for analysis.

Venous blood was analyzed to estimate the levels of AST, ALT, and ALP by TRIS buffer method, total protein by biuret method, total bilirubin by diazo dichloro aniline reagent method, LDH by p-l Scandinavian method and PT using the lyoplastin-LS reagent. The results obtained were compared between the cases and controls. The results were also compared with the APGAR score and the HIE stage of the cases to determine the utility of hepatic enzymes in determining the severity of asphyxia and its relation to severity.

Ethical clearance from IEC (Institutional ethics committee) had been taken before starting study.

## RESULTS

### Demographic profile

The mean birth weight of cases in our study was  $2.79 \pm 0.8$  kg and for controls it was  $3.14 \pm 1.3$  kg. Among the cases, 52.1% were female and 47.9% were male. Among the controls, 41.7% were male and 58.3% were female. Mean gestational age in our study was  $37.4 \pm 0.9$  and  $38.3 \pm 0.6$  weeks among cases and controls respectively (Table 1).

**Table 1: Distribution of cases and control.**

Total	Male (%)	Female (%)	Birth weight	Gestational age (Weeks)
<b>Case (115)</b>	47.8	52.1	$2.79 \pm 0.8$	$37.4 \pm 0.9$
<b>Control (115)</b>	41.7	58.3	$3.14 \pm 0.6$	$38.3 \pm 0.6$

### Cases

The proportion of neonates having severe birth asphyxia (Apgar score of 3 or less at 1 minute) in the present study was 36.5%, while neonates having moderate birth asphyxia (Apgar score of 4-6 at 1 minute) in the present study was 63.4% (Table 2).

**Table 2: Type of birth asphyxia.**

Total	Moderate birth asphyxia (%)	Severe birth asphyxia (%)
<b>Cases (115)</b>	73 (63.4)	42 (36.5)

Among the cases with HIE, 41.7% had HIE stage 1, 43.9% had HIE stage 2, while 15.6% developed HIE stage 3 (Table 3).

**Table 3: Proportion of different stages of HIE among cases.**

Total	HIE stage 1 (%)	HIE stage 2 (%)	HIE stage 3 (%)
<b>Cases (115)</b>	41.7	43.9	15.6

**Table 4: Liver function test values in cases and control.**

Variables	AST	ALT	LDH	ALP	PT	Total protein	Total bilirubin
Cases	80.3±32.3	53.5±29.1	842.2±493.9	224.4±82.9	16.6±1.8	5.4±0.9	8.6±2.7
Control	36.7±19.5	23.4±12.3	484.4±243.9	159.5±37.5	16.2±1.7	5.0±0.9	9.7±3.0

**AST**

The mean level of AST in the present study among cases was 80.3±32.3 and among controls was 36.7±19.5. The difference was statistically significant ( $p<0.05$ ) as shown in the Table 4.

**ALT**

ALT levels were 53.5±29.1 in cases and 23.4±12.3 in controls (Table 4).

**LDH**

The mean LDH levels between cases and control in the present study (842.2±493.9), (484.4±243.9) respectively were observed to have statistically significant difference (Table 4).

**ALP**

In this study, statistically significant difference was seen in the mean levels of serum ALP between cases (224.4±82.9) and control group (159.5±37.5) as shown in the Table 4.

**Prothrombin time**

It was 16.6±1.8 in cases and 16.2±1.7 in controls with difference being statistically significant as shown in the Table 4.

**Total protein and bilirubin**

Total protein was (5.4±0.9) in cases and (5.0±0.9) in controls. Total bilirubin was (8.6±2.7) in cases and (9.7±3.0) in controls, difference in both parameters was statistically insignificant (Table 4).

**Severity of birth asphyxia**

On the day three, within the cases, there was no statistically significant difference observed in the mean levels of AST, ALT, ALP, LDH, total protein, albumin, total bilirubin and prothrombin time of neonates with severe birth asphyxia (APGAR score of three or less at one minute) and neonates with moderate birth asphyxia (APGAR score of four to six at 1 minute). Hence it can be concluded that alteration in hepatic enzymes is not significant in relation to the severity of birth asphyxia based on the APGAR scores as shown in the Table 5.

**Different stages of HIE**

Using the post hoc test of inter-group analysis, it was found that the difference in AST, ALT and LDH was statistically significant between hypoxic ischemic encephalopathy stage 1 and stage 2 ( $p$  value of <0.5), between hypoxic ischemic encephalopathy stage 1 and stage 3 ( $p$  value of <0.5) but insignificant between hypoxic ischemic encephalopathy stage 2 and stage 3 ( $p$  value of >0.5). A rising trend was observed in serum levels of AST, ALT and LDH with the increase in hypoxic ischemic encephalopathy stage from 1 to stage 2 but not between stage 2 and stage 3 in the present study. Using post hoc test for intergroup analysis, it was also found that there was significant difference in the mean ALP levels within the 3 stages of hypoxic ischemic encephalopathy and the mean ALP levels showed an increasing trend with the increase in severity of hypoxic ischemic encephalopathy. There was no statistical significance in the mean prothrombin time when compared between different stages of hypoxic ischemic encephalopathy by using ANNOVA test and there was no increasing or decreasing trend found with the increasing severity of hypoxic ischemic encephalopathy as shown in the Table 6.

**Table 5: Comparison of hepatic function with severity of birth asphyxia.**

Hepatic function	Severity of birth asphyxia	Mean	P value
AST (U/L)	Severe	79.7±30.2	0.93
	Moderate	80.3±33.2	
ALT (U/L)	Severe	50.4±29.6	0.49
	Moderate	54.6±28.9	
ALP (U/L)	Severe	236.2±83.4	0.32
	Moderate	219.3±82.6	
LDH (IU/L)	Severe	818.1±504.6	0.74
	Moderate	851.7±492.7	
Total protein (g/dl)	Severe	5.4±0.93	0.92
	Moderate	5.4±0.94	
Albumin (g/dl)	Severe	2.9±0.58	0.67
	Moderate	2.8±0.63	
T. bilirubin (mg/dl)	Severe	8.58±1.9	0.97
	Moderate	8.6±2.93	
PT (seconds)	Severe	16.7±1.7	0.38
	Moderate	16.4±1.8	

**Table 6: Multiple comparison of AST, ALT, ALP, LDH and PT levels with different stages of HIE using post HOC test.**

Mean hepatic enzymes levels	Comparison between HIE stages	Mean difference	P value
AST	Stage 1 and 2	-38.29	<0.05
	Stage 1 and 3	-61.41	<0.05
	Stage 2 and 3	-23.11	0.3
ALT	Stage 1 and 2	-28.29	<0.05
	Stage 1 and 3	-51.42	<0.05
	Stage 2 and 3	-23.13	0.73
LDH	Stage 1 and 2	-670.46	<0.05
	Stage 1 and 3	-985.5	<0.05
	Stage 2 and 3	-315.05	0.53
ALP	Stage 1 and 2	-85.07	<0.05
	Stage 1 and 3	-128.76	<0.05
	Stage 2 and 3	-43.69	<0.05
PT	Stage 1 and 2	-1.63	0.24
	Stage 1 and 3	-1.72	0.18
	Stage 2 and 3	-0.08	0.92

## DISCUSSION

The present study was conducted to compare serum levels of hepatic enzymes between full term neonates with and without perinatal asphyxia, to study the alteration of hepatic function due to perinatal asphyxia. The alteration of these hepatic enzymes was also studied in relation to severity of perinatal asphyxia and with different stages of HIE.

It has been observed in previous studies that the values of the liver enzymes rise significantly at twelve hours but peak at about 24 to 72 hours before declining. The increase in the levels of the enzymes is substantial and are sustained for as long as 7-10 days after hypoxic injury.<sup>3</sup> The time of sample collection could therefore account for the wide variation in levels of AST, ALT and LDH in babies with perinatal asphyxia and that it was an evidence of liver injury. Islam et al collected the samples within 2<sup>nd</sup> and 5<sup>th</sup> days of life while Tarcan et al collected samples within twenty-four hours of life.<sup>10,11</sup> In the present study, the samples were taken at 72 hours when the levels of the enzymes had peaked.

The mean level of AST in the present study among cases was  $80.3 \pm 32.3$  and similar results were found for the mean AST levels in studies conducted by Choudhary et al, Kariya et al and Islam et al. Mean level of ALT in the present study among cases was  $53.5 \pm 29.1$  and similar results were found for the mean ALT levels in studies conducted by Choudhary et al, Kariya et al and Islam et al. A rising trend was observed in serum levels of AST, ALT and LDH with the increase in HIE stage from 1 to stage 2 but not between stage 2 and stage 3 in the present study. According to the study by Choudhary et al, a similar trend of increase in serum levels of AST and ALT was

observed with the increase in severity of HIE. Study conducted by Rajeesha et al also found similar results as of the present study and established an increasing trend in AST, ALT and LDH levels with increasing severity of HIE.<sup>5,10,12</sup>

The mean LDH levels between cases and control in the present study ( $842.2 \pm 493.9$ ), ( $484.4 \pm 243.9$ ) respectively were observed to have statistically significant difference. Reddy et al noted that LDH had 100% sensitivity when done at 72 hours of life with a maximum area under ROC curve in asphyxiated neonates.<sup>14</sup> Thus, it can be concluded that raised serum LDH levels at 24 to 72 hours of life may be indicative of hypoxic insult due to birth asphyxia. In this study, statistically significant difference was seen in the mean levels of serum ALP between cases and controls which was also seen in studies by Choudhary et al and Islam et al. It was also found that there was significant difference in the mean ALP levels within the 3 stages of HIE and mean ALP levels showed an increasing trend with the increase in severity of HIE. This finding was supported by the study conducted by Islam et al.<sup>5,10</sup>

For the serum levels of total protein and total bilirubin, the present study as well as the study conducted by Choudhary et al found no statistically significant difference between the cases and the control.<sup>5</sup> A large scale multicentric study is recommended to establish the relation between total protein, total bilirubin and prothrombin time in neonates with birth asphyxia.

The enzyme levels did not correlate well with the severity of perinatal asphyxia (based on APGAR score) at this age as the difference in the enzyme levels between full term neonates with severe and moderate birth asphyxia was statistically insignificant. The reason might be due to a small sample size, single center study with hospital population, so result could not be generalized for the whole population. We have not included the use of cord blood pH in determining severity of birth asphyxia.

## CONCLUSION

The present study showed that the serum levels of hepatic enzymes were higher in full term neonates with perinatal asphyxia than full term neonates without perinatal asphyxia at the age of 72 hours. The enzyme levels showed an increasing trend with increasing severity of HIE. Assaying the hepatic enzymes may facilitate early suspicion of perinatal asphyxia in full term neonates who can be provided with neuroprotective interventions to improve the outcome especially in a simple health care set up with minimum diagnostic back up.

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