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A comparative study of liver function test between normal neonates and neonates with different stages of hypoxic ischemic encephalopathy in a district medical college of Eastern India

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ABSTRACT

Background: Hepatic dysfunction is a known complication in neonates with hypoxic ischemic encephalopathy (HIE). **Aims and Objectives:** We conducted this study to compare hepatic function between normal and asphyxiated newborn and whether the changes in liver function vary with the grade of HIE. **Materials and Methods:** This observational analytical cross-sectional study was done in a rural medical college of eastern India for 1 year with 51 HIE (17 each in HIE I, II, and III group) and 51 healthy neonates as control. Biochemical parameters of liver function such as alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), total serum bilirubin (TSB), serum albumin, and prothrombin time (PT) were measured in both groups between 48 and 72 h of birth. **Results:** We found that TSB, liver enzymes, and PT ware significantly elevated and serum albumin reduced in HIE group in comparison to control group. However, TSB, PT, and serum albumin does not differ much among three stages of HIE. By contrast, liver enzymes increases significantly among three stages of HIE, and also, there is significant difference in ALT, AST, and ALP between survivor and non-survivor of HIE babies. **Conclusion:** Hence, liver function test may serve as an useful early marker of perinatal asphyxia when the history of birth asphyxia is lacking and it can also be used as a prognostic indicator as there is more rise of liver enzymes from low to high grade of HIE and from survived to expired HIE babies.

Key words: Hypoxic ischemic encephalopathy; Liver function test; Neonate

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INTRODUCTION

Perinatal asphyxia is one of the leading causes of neonatal mortality all over the world including the developing countries like India. Perinatal asphyxia, leading to hypoxic brain injury and neonatal encephalopathy, is called hypoxic ischemic encephalopathy (HIE). This HIE not only causes immediate mortality but also affect the survived neonates with long-term morbidity such as recurrent seizure, cerebral palsy, mental retardation, or other neurodevelopmental delay. In addition to the hypoxic-ischemic brain injury and neurological deficits, there is evidence of multi-system insult to the newborn, involving heart, lungs, kidneys, liver, gastrointestinal system, and bone marrow. The outcome of asphyxiated babies depends on severity of hypoxia which adversely affects the normal functions of the multiple organs of the newborn. In response to the hypoxic ischemic, insult body tries to save its vital organs such as brain, heart, and adrenal at the expense of other relatively non-vital

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organ like gastrointestinal tract, kidneys, liver, and spleen by redistribution of cardiac output from non-vital to vital organ. In this process of saving vital organs, liver is affected by hypoxic ischemic injury and which causes derangement of its normal metabolic and synthetic functions. As a result of that, liver enzymes such as alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP) show a rapid and temporary elevation and synthesis of serum albumin and vitamin k-dependent clotting factors may be impaired as well. These changes in liver function tests (LFT) not only help as a biochemical marker of perinatal asphyxia but also help to detect the severity of asphyxia. Although there are many studies across the world about liver function test in asphyxiated babies, there is lack of data from any rural medical college of eastern India. Hence, we performed this study to evaluate hepatic dysfunction in perinatal asphyxia and to assess the severity of hepatic involvement with the severity of asphyxia.

Aims and objectives

The specific objectives of this study were as follows; 1) To find out any changes in LFT including ALT, AST, ALP, total serum bilirubin (TSB), serum albumin, and prothrombin time (PT) in HIE babies in comparison to normal newborns and 2) whether the changes in liver function test varies with the severity of HIE (from Grade I to III) or differ significantly between survived and non-survived HIE neonates.

MATERIALS AND METHODS

After developing parameters and indicators and taking necessary permission from the concerned authority and Institutional Ethics Committee (IEC) of the college, this observational analytical cross-sectional study was carried out at the sick newborn care unit of a tertiary care hospital of west Bengal, India for an duration of 1 year. During this 1 year study period, we planned to take equal number of babies in HIE I, II, and III grade and also same no of normal newborn with that of the total HIE babies for comparison. Hence, we started selecting HIE newborns by convenient sampling method and continue our sampling until our desired no of HIE newborns that were included (HIE I –17, HIE II – 17, and HIE III – 17, and Total – 51) and in the process, we deliberately excluded some HIE I and HIE II cases to get equal no in three groups. Thus, our study group comprises 51 newborns suffering from birth asphyxia and subsequently developing HIE fulfilling American academy of pediatrics and American College of obstetrics and gynecology criteria.[1] Here, we have categorized newborns into HIE I, II, and III according to modified Sarnat and Sarnat HIE staging.[2] We included newborns suffering from HIE fulfilling the following inclusion and exclusion criteria.

Inclusion criteria

The following criteria were included in the study:

1. Gestational age between 37 and 42 weeks
2. Birth weight ≥2.5 kg and
3. Age within 72 h of birth.

Exclusion criteria

The following criteria were excluded from the study:

1. Newborns with sepsis
2. Major congenital malformations
3. Primary disease of the hepatobiliary system
4. Hemolytic disease and neonatal jaundice
5. Newborns born to mother with history of intake of hepatotoxic drug.

Standard treatment for HIE was given to all the included newborns during the study. Control group of 51 healthy newborn is selected from healthy neonates from postnatal ward with the same inclusion and exclusion criteria by matching for gestational age and birth weight. First, the parents were interviewed regarding the antenatal history, drug history of mother, etc., and newborns were examined for any major congenital malformations, hemolytic disease, sepsis, liver disease, etc. Then, the parents of the newborns eligible for the study as per inclusion and exclusion criteria were briefed regarding the purpose and procedure of study. A written consent was obtained from them regarding this matter. The eligible newborns were screened for other conditions mimicking HIE such as hypoglycemia and hypocalcaemia. A thorough clinical examination and observation were done and subjects were categorized according to modified Sarnat and Sarnat HIE staging. Five ml of venous blood were collected aseptically from each newborn between 48 and 72 h of their birth and the collected blood samples were sent to the Department of Biochemistry for estimation of AST, ALT, ALP, serum albumin, TSB, and PT. USG abdomen was done to exclude any congenital malformation or abnormality of the hepatobilliary system. All HIE babies who developed liver dysfunction were treated according to the protocol and number of babies expired in different groups of HIE ware noted.

Statistical analysis

The collected data are entered in Microsoft Excel worksheet (Microsoft, Redwoods, WA, USA) and appropriate statistical tests are used for analysis such as Chi-square and student's t test. All the statistical analysis are done in SPSS software, version 21.0 (the Statistical Package for the Social Sciences Inc, Chicago, IL, USA) and the data are assessed for statistical significance at $P<0.05$.

RESULTS

In this study, we have done LFT including PT in 51 HIE neonates and 51 normal neonates with matched birth weight and gestational age. Table 1 shows that the TSB level was increased in the study group (6.02±0.81) in comparison to reference group (5.00±0.80); P value being less than 0.001. ALT was 84.74±11.23 U/L in comparison to 27.58±5.37U/L in control group, which was statistically significant (P<0.001). AST was 75.98±11.34U/L in study group and 23.15±5.07U/L in control group, the difference being significant (P<0.001). Similarly, ALP was significantly high in study group (364.64±60.18 U/L) in comparison to control group (197.68±31.19U/L) with P<0.001. The albumin level was also low in the study group in comparison to reference group (3.22±0.41 g/dl vs. 4.10±0.42 g/dl, P<0.001). The difference in PT between the two groups is also statistically significant (P<0.05).

In Table 2, we have compared the mean value of LFT and PT between three stages of HIE. ALT was significantly higher in HIE III group (93.35±6.86 U/L) in comparison to HIE II (86.59±8.71 U/L) and HIE I group (74.29±8.55 U/L). We found AST 88.53±4.57 U/L in HIE III group, 75.29±6.04 U/L in HIE II group, and 64.12±5.23 U/L in HIE I group with statistically significant difference (P<0.001). Similarly, ALP was also higher in higher HIE grade (407.94±56.42 U/L in HIE III, 363.24±51.11 U/L in HIE II, and 322.76±40.79 U/L in HIE I) with P<0.001. The difference of TSB, serum albumin, and PT between Stages I, II, and III HIE was not significant statistically.

Table 3 shows how mortality increases with advance stage of HIE. Total eight neonates expired in the study group out of 51 (15.69%). In HIE I group, all neonates survived. Mortality is 17.65% in HIE II and 29.42% in HIE III group. When we compare between any two stage (using Chi-square test for comparison of proportion), the difference between Stages I and II is statistically significant (P=0.0018), but it is not significant between Stages II and III (P=0.1633).

In Table 4, we have compared the three parameters ALT, AST, and ALP among the survived and expired neonates. In the study group, ALT is significantly higher among the expired (98.13±3.90 U/L) in comparison to the survived neonates (82.23±10.34 U/L). AST is 88.13±6.01 U/L among expired and 74.26±11.07 U/L among survived neonates with P<0.001. There is also significant difference in ALP value among expired (430.38±20.59 U/L) and survived (356.37±59.13U/L) neonates (P<0.001).

DISCUSSION

It is a well-known fact that perinatal asphyxia not only affect the brain but also other organ system of the newborn including liver.[3,4] Hypoxic injury of the liver frequently alters its metabolic and synthetic function and manifested with deranged LFT. In HIE affected neonates, hepatic function of bilirubin metabolism is hampered and serum bilirubin level rises. In the present study, TSB is significantly higher in the HIE babies as compared to normal control. Similar findings were described by the study of Islam et al., and Choudhury et al.[5,6]

Another important parameter is liver enzyme such as ALT, AST, and ALP which are normally situated inside the liver cells, but when the liver is injured by hypoxia that

Table 1: Comparison of LFT between study group (HIE cases) and control group (normal neonates)

LFT	Study group (N=51) Mean±SD	Control group (N=51) Mean±SD	P value
Total Serum Bilirubin (TSB) (mg/dl)	6.02±0.81	5.00±0.80	<0.001
ALT (U/L)	84.74±11.23	27.58±5.37	<0.001
AST (U/L)	75.98±11.34	23.15±5.07	<0.001
ALP (U/L)	364.64±60.18	197.68±31.19	<0.001
Serum albumin (g/dl)	3.22±0.41	4.10±0.42	<0.001
Prothrombin Time (s)	15.10±1.40	14.70±1.11	<0.05

LFT: Liver function tests, ALT: Alanine transferase, AST: Aspartate transferase, ALP: Alkaline phosphatase

Table 2: Comparison of LFT between stages of HIE in the study group

LFT	HIE Stage I (N=17) Mean±SD	HIE Stage II (N=17) Mean±SD	HIE Stage III (N=17) Mean±SD	P value
Total serum bilirubin (TSB) (mg/dl)	6.12±0.85	6.00±0.79	5.94±0.82	>0.05
ALT (U/L)	74.29±8.55	86.59±8.71	93.35±6.86	<0.001
AST (U/L)	64.12±5.23	75.29±6.04	88.53±4.57	<0.001
ALP (U/L)	322.76±40.79	363.24±51.11	407.94±56.42	<0.001
Serum Albumin (g/dl)	3.25±0.45	3.20±0.37	3.20±0.42	>0.05
Prothrombin Time (s)	14.88±1.72	15.29±1.35	15.12±1.11	>0.05

TSB: Total serum bilirubin, LFT: Liver function tests, ALT: Alanine transferase, AST: Aspartate transferase, ALP: Alkaline phosphatase

Table 3: Outcome in the study group according to the HIE stage

HIE stage	Total neonates	Expired neonates	Mortality percentage within each stage (%)
HIE I	17	0	0
HIE II	17	3	17.65
HIE III	17	5	29.42

HIE: Hypoxic ischemic encephalopathy

Table 4: Comparison of liver enzymes between survived and expired neonates in the study group

Biochemical parameters	Survived (N=43) Mean±SD	Expired (N=08) Mean±SD	P value
ALT (U/L)	82.23±10.34	98.13±3.90	<0.001
AST (U/L) T	74.26±11.07	88.13±6.01	<0.001
ALP (U/L)	356.37±59.13	430.38±20.59	<0.001

ALT: Alanine transferase, AST: Aspartate transferase, ALP: Alkaline phosphatase

they are spilled into the bloodstream. Among them, ALT is more specific of liver injury as liver is the main source of this enzyme. When a newborn is affected by birth asphyxia, these enzymes rise sharply in the first 24–72 h of life and again returns to normal after 10 days of life in most cases.[7] Islam et al., in their study, in 2011, found that mean AST, ALT, and ALP of the asphyxiated babies were 76.27±37.44, 82.16±48.08, and 369.59±123.05 U/L and that of normal babies were 23.46±8.45, 26.54±7.76, and 208.20±46.95 U/L, respectively, and these rise were statistically significant (P<0.001).[5] The levels of AST, ALT, and ALP were positively correlated with the severity of asphyxia and these correlations were also statistically significant (P<0.001). Our observation is in close approximation to those reported by Islam et al. Study of Paliwal et al., also showed that serum AST and ALT were significantly higher in asphyxiated babies in comparison to control group.[8] In our study, we have found that ALT, AST, and ALP level were showing increasing trend with the advancement of HIE stage and the difference is statistically significant. The study of Choudhury et al., also shows the rising trend of ALT and AST with the progression of newborns from HIE Stage 1(mean ALT 39±22.99U/L and AST 70±35.81U/L) to HIE Stage III (mean ALT 74.75±31.44 U/L& AST 157.25±0.25 U/L).[6]

As liver is the major organ for the production of Vitamin K-dependent blood coagulation factors, so hepatic damage by hypoxia is manifested by derangement of blood coagulation system. Defect in the extrinsic pathway of coagulation causes prolonged PT in HIE-affected neonates. In our study, also PT is significantly higher in HIE group as compared to control which is in accordance to some previous study like Elsadek et al., study, where they have found PT and INR significantly higher in HIE babies.[9] Serum albumin is also a marker of hepatic integrity and it is decreased when the synthetic function of liver is hampered by hypoxic ischemic damage. In our study, we have found serum albumin significantly low in HIE group in comparison to the control group. This finding is similar to other studies like Elsadek et al., or Choudhury et al., where they described total protein and serum albumin significantly low in babies with perinatal asphyxia.[6,9]

In the present study, levels of ALT, AST, and ALP are significantly higher among different stages of HIE, from mild to severe. However, TSB, PT, and serum albumin did not have any significant difference with increasing severity of HIE. This finding is supported by some studies like Islam et al., and contradicted by some like the Elsadek et al., study, where they found no significant correlation between serum level of ALP and stages of HIE.[9-11] In our study, we have shown that how mortality is increased from lower to higher HIE stages (nil in HIE I to 29.42% in HIE III). Study by Sharma et al., showed also that there is a significant increase in mortality in neonates with severe asphyxia in comparison to mild or moderate asphyxia group.[12]

The present study revealed that level of all three liver enzymes is higher among the expired neonates in comparison to the survived HIE babies and the difference is statistically significant. Saili et al., in his study, also found that the serum level of ALT, AST, and ALP was significantly higher in expired group than those of survived group of newborns.[13] Hence, greater degree of elevation of liver enzymes might be a poor prognostic factor for the HIE affected neonates as described by other studies also.[14-17]

Limitations of the study

This is a single-center study with small number of sample. A multicenter study with larger sample size is needed before drawing a conclusion that we can really depend on liver function test as a diagnostic and prognostic tool in neonates with perinatal asphyxia and HIE.

CONCLUSION

This study shows that the liver enzymes, TSB and PT, are elevated and serum albumin decreased in the neonates suffering from HIE. Hence, we can use liver function test as a supporting evidence of perinatal asphyxia when a neonate presented with features of HIE without a proper history of birth asphyxia. Elevated liver enzymes not only point toward perinatal asphyxia when other causes are excluded but it also indicates about the severity of asphyxia. Thus, more elevated the enzyme is, more the chance that the baby is in higher HIE grade and in higher risk of mortality.

Therefore, deranged hepatic function in a HIE baby may serve as a prognostic tool also to predict the outcome of the baby and can alert the treating pediatrician to give more aggressive management to save the baby.

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KLB- Critical revision of the manuscript; **BP, AA-** Concept and design of the study, review of literature; **NG, SB-** Data acquisition, statistical analysis; **SL-** Manuscript writing, manuscript editing

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