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Level of Serum Lactate Dehydrogenase, Creatine Kinase And Uric Acid As Predictors of Hypoxic Ischemic Encephalopathy in New Born Infants with Birth Asphyxia

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Abstract: *Hypoxic-ischemic encephalopathy (HIE) due to birth asphyxia is an important etiology of neonatal morbidity and mortality and is conventionally diagnosed on the basis of clinical features and promptly classified by various classifications and treated accordingly. By this protocol, often the cases are diagnosed late and this leads to increased mortality and morbidity. The aim of this study was to investigate the predictive values of biochemical parameters, like serum creatine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA) in newborns with HIE. A total of 42patients were diagnosed with HIE by Sarnat and Sarnat classification, the Levene's classification and the Thompson scoring and 50 healthy newborn as controls were enrolled into the study. The results obtained showed that there is statistically significant increase in levels of LDH, CK, CK-MB and UA within first 12 to 24 hours in an asphyxiated new born infant suffering from HIE 1 compared to a normal subject. However, with progress to HIE 2 from HIE 1 only level of UA show a statistically significant change. Measurement of serum CK, LDH, lactate, and UA levels is a promising method in determining the stage of hypoxia before clinical manifestations occur so that hypothermia treatment can be initiated earlier.*

Keywords: *hypoxic-ischemic encephalopathy, creatine kinase, lactate dehydrogenase, uric acid.*

I. Introduction

Birth asphyxia or perinatal asphyxia[1] is a condition of impaired gas exchange within the fetus, gradually leading to a state of fetal hypoxemia and acidosis. Prolonged hypoxic conditions in new born infants as a result of birth asphyxia will lead to impairment of blood flow to vital organs like the brain leading to brain injury which can manifest itself as a neurobehavioral state known as Hypoxic Ischemic Encephalopathy (HIE). Hypoxic damage can occur to most of the infant's organs (heart, lungs, liver, gut, kidneys etc.). But brain damage is of most concern and perhaps the least likely to quickly or completely heal.The incidence of perinatal asphyxia is approximately 1% to 1.5% in the western countries and accounts for 20% perinatal deaths in India and worldwide. [1] The damage can occur in the cerebral cortex, basal ganglia, hypothalamus as focal or diffused lesions manifesting themselves clinically according to the site of involvement as changes in level of consciousness, muscle tone, papillary changes, seizures, etc. Diagnosis[1] of Perinatal asphyxia is done by risk estimation, persistant low APGAR score of less than 3 for more than 5 minutes, prolonged metabolic or mixed academic pH of <7.0 on cord or arterial blood sample, Blood flow to the brain is maintained during systemic hypotension by an auto regulatory mechanism, which is impaired in asphyxial conditions and hence decreased perfusion to brain leading to ischemia, which causes excitotoxicity [2] leading to neurological manifestations like seizures, hypotonia, etc, and clinical evidence of multi organ failure like ventilatory dependence, necrotizing enterocolitis, shock, etc.

We classify HIE by several methods of classification and scoring like the Sarnat and Sarnat [3] classification, the Levene's classification [4] and the Thompson scoring[5], all of which are based on the clinical features within the immediate neonatal period. But for neurological manifestations to appear, it can take upto 72 hours [6] . By the Sarnat staging, the outcome varies with the severity. Stage 1 HIE, 98% or more will have a normal neurological outcome with <1% mortality. Stage 2 HIE, 20% to 37% die or have abnormal neurological outcome. In case of stage 3 HIE, 50% to 89% patients die and all of them have abnormal neurological manifestations. Management of HIE for each of these stages is done promptly after classification and staging. But by then, most of the damage has already set in.

Several studies have been carried out to try and correlate biochemical markers of perinatal asphyxia with its neurological sequelae and stages of HIE. In studies were conducted by Reddy S, et al in 2008[7]; Karlsson M et al, in Aug 2010[8] and in 2012[9] again it was found that these biomolecules, especially LDH show significant rise in the initial perinatal period. Fernandez et al.[10]and Sweet et al. [11] measured the serum CK-BB (brain isoenzyme) activities of 33 full-term newborns in the 4th and 10th hours of life and on 97 newborns with asphyxia respectively found that infants who died of severe HIE or developed neurological abnormalities while they were being monitored had significantly higher serum CK-BB activities than infants who did not develop

neurological anomalies. Based on their study, Fernandez et al. claimed that a high serum CK-BB activity is a sensitive marker of brain injury whereas Sweet et al. concluded that CK-BB is not a useful marker depending on sensitivity and specificity in the prediction of the neurological results of perinatal asphyxia . Adhikari et al. [12] showed that 30 newborns with asphyxia (16 severe, 14 moderate) continued to have cerebral edema for more than 48 hours as revealed by cranial ultrasonography.. On the other hand, Serdar Beken et al.[13] in their study preferred to measure total CK levels instead of the levels of the brain-specific isoenzyme of CK because, even though Sarnat and Sarnat staging system is based mainly on the functions of the nervous system, it also involves other systems. Their study reveals the noteworthy fact that serum total CK levels have a low specificity but high sensitivity in determining the HIE stage. They also found statistically significant increase of serum lactate,UA and LDH levels with regard to the stages of HIE. So, they concluded that the serum levels of LDH, UA and CK, conducted routinely before the clinical determination of the HIE stage in newborns with perinatal asphyxia ..

The only medical treatment with promising long-term neurodevelopment results, is therapeutic hypothermia. However, this treatment is effective only if it is administered in the early post natal period. Thus biochemical markers that can be used in determining the HIE stage before clinical manifestations of the disease appear will be of great help.

So in our study with the above context of varied opinions we are going to investigate if any correlation of Lactate Dehydrogenase (LDH), Creatine Kinase (CK) and Uric Acid (UA) levels during the first 24 hours after birth with the occurrence of different stages of HIE is present or not and how they are related with different stages of HIE in this part of our country in order to be able to use these enzymes as markers or predictors of HIE before the appearance of significant clinical features to allow earlier management of HIE and improve the mortality and morbidity rates due to it.

II. Materials and Methods

It is a Cross sectional observational hospital based study done at Department of Gynaecology and Obstetrics, R. G. Kar Medical College and Hospital, Kolkata; Sick Neonatal Care Unit (SNCU) of Department of Paediatrics, R. G. Kar Medical College and Hospital, Kolkata and Department of Biochemistry, R. G. Kar Medical College and Hospital, Kolkata. Cases and controls are the new born infants delivered in Department of Obstetrics and admitted to the Sick Neonatal Care Unit, R. G. Kar Medical College and Hospital, Kolkata.

New born infants with signs of birth asphyxia will be taken as the target sample depending on these Inclusion Criteria such as persistence of an APGAR score [14] 0–3 for more than 5 minutes , clinical evidence of multiorgan system dysfunction like oligo-anuria, congestive heart failure not related to structural defects, shock, ventilatory dependence or requirement of increased oxygen in more than 24 hours, elevated transaminases, DIC, necrotising enterocolitis, etc. Patients are Excluded with a gestational age of <36 weeks, with major congenital malformation, chromosomal abnormalities, metabolic disorders, congenital infection, birth trauma, and septic shock were excluded from our study. For the control blood of 50 new born infants with no sign of birth asphyxia taken from their mother's cord blood at the time of delivery. The study is performed with the approval of the Institutional Ethical Committee (IEC) and proper consent from the patient party. A baseline clinical picture of the subjects was collected during the time of blood collection. Additional data was acquired from the records kept at the department later on. The subjects were classified on the basis of the clinical features by a modification of the Thompson score and the Levene's classification, as HIE 1, 2 (there was no HIE 3 patient in the study as they were critically ill and hence blood collection was problematic).

The biochemical analysis for the parameters, that is LDH, CK-NAC, CK-MB, UA were done using reagent kits and Auto Analysers and Semi Auto Analysers.

2.1. Estimation of LDH by Lactate to Pyruvate method of Gay, Bowers and Mc Combs (1968) [15,16,17] .

1ml of working reagent Pipetted into test tubes and 0.05ml of sample. These are mixed and absorbance is read at 1 minute and thereafter at 30, 60, 90 seconds at 340 nm. The mean change in absorbance per minute is determined to calculate the test results. The method is linear upto 2000 IU/L.

2.2 Estimation of CK [18]

working reagent 1 ml and sample 0.05 ml was mixed well. Initial absorbance is read at 1minute and read after every 1, 2, 3 minutes thereafter. The mean absorbance change per minute is calculated.The procedure is linear upto 2000 U/L at 37 degree.

2.3. Esmtionf CK-MB [18]

Working reagent 1 ml was mixed with sample 0.05 ml and initial absorbance read at 10 minutes. Readings are taken again after every 1, 2, 3 minutes to calculate the mean absorbance change per minute. The procedure linear upto 1000 U/ml.

2.4. Estimation of Uric Acid Level Uricase or PAP Method [19,20]

Working reagent is prepared by mixing of Buffer reagent, Enzyme reagent. Working reagent 1 ml was mixed with 0.02ml of sample and the mixtures are incubated at 37^0 for 5 minutes. The absorbance of the test was measured within 30 minutes.This procedure is linear upto 20 mg/dl.

III. Results

Data was recorded and analyzed using the Statistical Package for the Social Sciences (SPSS) 20.0.Table 1 shows the distribution of the study population as the asphyxiated new born infants diagnosed to have HIE as opposed to the non asphyxiated group of new born infants.

Table 1

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Hie	42	45.7	45.7	45.7
	Non Asphyxiated	50	54.3	54.3	100.0
	Total	92	100.0	100.0	

Table 2 shows distribution of study population according to staging or classification.

Table 2

		Frequency	Percent	Valid Percent
Valid	HIE 1	30	32.6	32.6
	HIE 2	12	13.0	13.0
	Non Asphyxiated	50	54.3	54.3
	Total	92	100.0	100.0

Descriptive statistics gives us the mean level of LDH in case of HIE baby to be 760.84 (SD 455.725) as opposed to the mean level of LDH in case of the normal non asphyxiated baby, which is 290.30 (SD 89.277) and is shown in the box whisker plot (Fig.1). Statistical analysis give the mean level of CK in case of HIE babies to be 1718.56 (SD 1557.439) as opposed to the mean level of CK in normal non asphyxiated babies which is 290.29 (SD 139.846) This is further demonstrated in the box whisker plot (Fig.2).

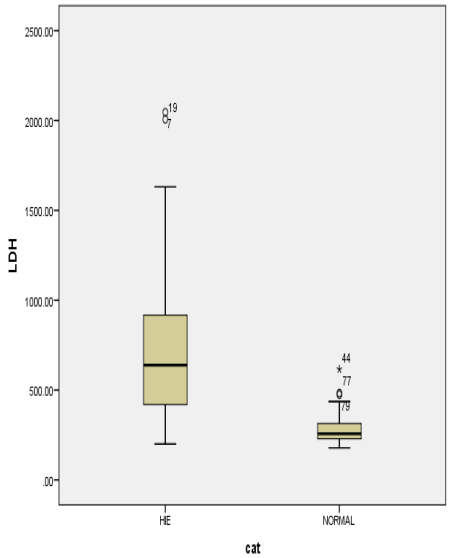


Figure 1

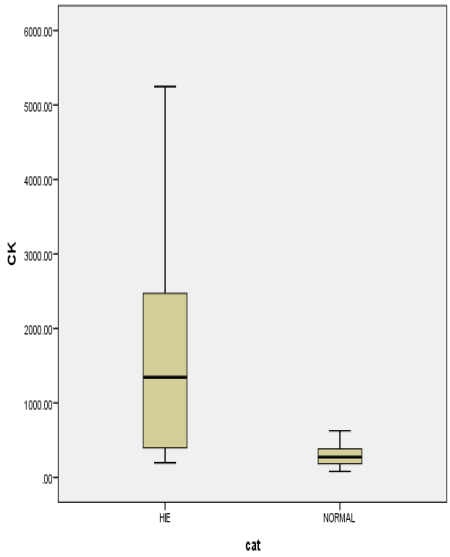


Figure 2

Statistical analysis give the mean level of CK-MB in case of HIE babies to be 145.28 (SD 115.766) as opposed to the mean level of CK-MB in normal non asphyxiated babies which is 57.76 (SD 65.133) .This is further demonstrated in the box whisker plot (Fig.3).

Statistical analysis give the mean level of UA in case of HIE babies to be 5.34 (SD1.808) as opposed to the mean level of UA in normal non asphyxiated babies which is 3.97 (SD 1.096) .This is further demonstrated in the box whisker plot (Fig. 4).

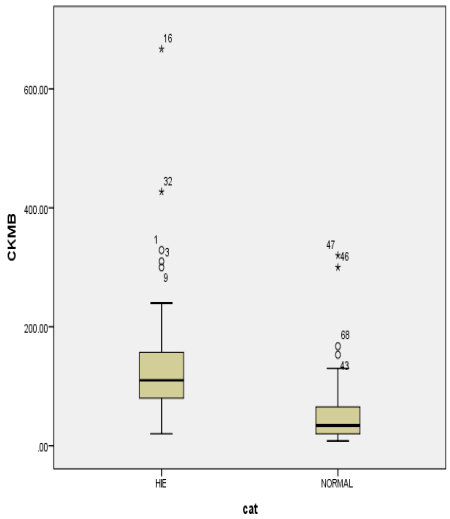


Figure 3

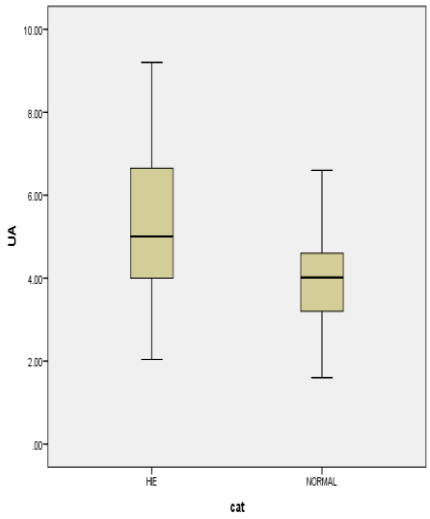


Figure 4

To test the significance of mean differences of various parameters, we have to know the distribution of the various parameters in the study. For this we check whether the parameters are normally distributed or not by the Shapiro Wilk Test. The test results as shown in TABLE 3 shows that only UA is normally distributed in the study population.

Table 3 Tests Of Normality

	Cat	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	Df	Sig.
Ua	Hie	.096	42	.200*	.979	42	.622
	Normal	.086	50	.200*	.979	50	.505
Ldh	Hie	.177	42	.002	.861	42	.000
	Normal	.187	50	.000	.848	50	.000
Ck	Hie	.164	42	.006	.841	42	.000
	Normal	.078	50	.200*	.958	50	.070
Ckmb	Hie	.283	42	.000	.694	42	.000
	Normal	.236	50	.000	.679	50	.000
*. This Is A Lower Bound Of The True Significance.							
a. Lilliefors Significance Correction							

Table 4

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
UA	Equal variances assumed	11.131	.001	4.466	90	.000
	Equal variances not assumed			4.288	65.017	.000

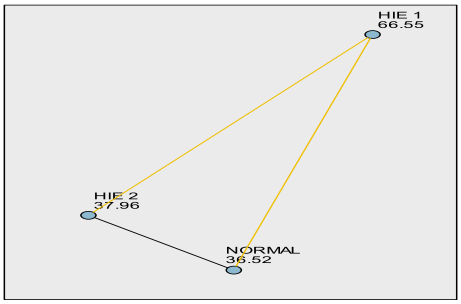
Now we have 30 case of HIE 1 and 12 cases of HIE 2. The mean LDH levels of HIE 1 and HIE 2 are respectively 748.98 (SD 479.18) and 790.47 (SD 409.15). The mean CK in HIE 1 cases is 1992.13 (SD 163.64) and HIE 2 is 1034.63 (SD 1143.62). The mean CK-MB in HIE 1 cases is 134.51 (SD 124.94) and HIE 2 cases is 172.22 (SD 89.69). The mean values of UA in cases of HIE 1 and HIE 2 cases are respectively 5.89 (SD 1.64) and 3.95 (SD 1.47) (TABLE 4). As the uric acid is normally distributed among the study subjects, for testing significance between the 3 groups that is HIE 1, HIE 2 and non asphyxiated normal babies, we can go for the Spearman correlation test and multiple comparison test. The test results by the Spearman correlation shows that there is significant difference of mean across groups (P= 0.000) (TABLE 5). Now, by multiple comparison, we see that there is significant difference of mean between HIE 1 and HIE 2, and HIE 1 and normal, with P=0.006 and P=0.000 respectively. However, there is no statistical significance of difference between HIE 2 and normal with P=1.000 (Fig.5).

Table 5 Group Statistics					
	cat	N	Mean	Std. Deviation	Std. Error Mean
UA	HIE	42	5.3379	1.80811	.27900
	NORMAL	50	3.9694	1.09564	.15495

The variables LDH, CK and CK-MB are not normally distributed in the study population. So we have to go for the non parametric tests in order to test the significance of the differences between their mean values.

Test of significance between HIE 1 and HIE 2 groups is done by Spearman's correlation test shows that there is statistically significant difference of mean of these 2 groups in case of CK (P=0.032), (Table 6) CK-MB (P=0.001) (TABLE 7) and Uric acid (P=0.002) (TABLE 8**)** levels, but there is no statistical significance for LDH (P=0.238). **(**TABLE 9**)**

Pairwise Comparisons of staging



Each node shows the sample average rank of staging.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
NORMAL-HIE 2	300.000	56.097	.000	.500	1.000
NORMAL-HIE 1	251.000	100.604	-4.960	.000	.000
HIE 2-HIE 1	77.500	35.906	-2.855	.002	.006

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (1-sided tests) are displayed. The significance level is .05.

Figure 5

For Ldh, Ck, Ck-Mb we do the independent Jonckheere Terpestra test. By this test, the pair wise comparison of various groups reveals that there is significant difference of mean values of normal and HIE 1 subjects, and normal and HIE 2 subjects in case of LDH, CK and CK-MB, but there is no significant difference between HIE 1 and HIE 2 subjects. This has been depicted in fig. 6 to fig. 8 respectively.

Table 6 Correlations				
			ck	staging
Spearman's rho	CK	Correlation Coefficient	1.000	-.289*
		Sig. (1-tailed)	.	.032
		N	42	42
	staging	Correlation Coefficient	-.289*	1.000
		Sig. (1-tailed)	.032	.
		N	42	42
*. Correlation is significant at the 0.05 level (1-tailed).				

Table 7

Correlations				
			ckmb	staging
Spearman's rho	CK-MB	Correlation Coefficient	1.000	.450**
		Sig. (1-tailed)	.	.001
		N	42	42
	staging	Correlation Coefficient	.450**	1.000
		Sig. (1-tailed)	.001	.
		N	42	42

**. Correlation is significant at the 0.01 level (1-tailed).

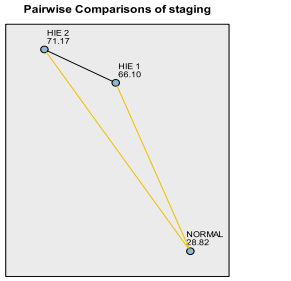
Table 8 Correlations

			ua	staging
Spearman's rho	UA	Correlation Coefficient	1.000	-.446**
		Sig. (1-tailed)	.	.002
		N	42	42
	staging	Correlation Coefficient	-.446**	1.000
		Sig. (1-tailed)	.002	.
		N	42	42

**. Correlation is significant at the 0.01 level (1-tailed).

Table 9 Correlations

			ldh	staging
Spearman's rho	LDH	Correlation Coefficient	1.000	.113
		Sig. (1-tailed)	.	.238
		N	42	42
	staging	Correlation Coefficient	.113	1.000
		Sig. (1-tailed)	.238	.
		N	42	42

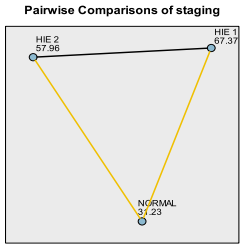


Each node shows the sample average rank of staging.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
NORMAL-HIE 1	136.000	100.622	-6.102	.000	.000
NORMAL-HIE 2	30.000	56.123	-4.811	.000	.000
HIE 1-HIE 2	206.000	35.915	.724	.765	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (1-sided tests) are displayed. The significance level is .05.

Figure 6: LDH

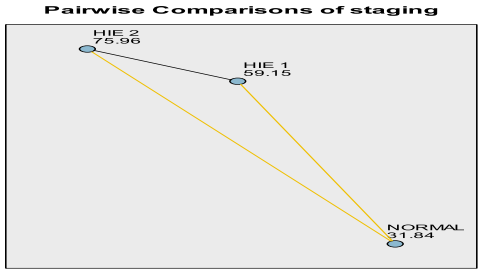


Each node shows the sample average rank of staging.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
NORMAL-HIE 2	96.000	56.125	-3.635	.000	.000
NORMAL-HIE 1	190.500	100.622	-5.560	.000	.000
HIE 2-HIE 1	113.500	35.914	-1.852	.032	.096

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (1-sided tests) are displayed. The significance level is .05.

Figure 7: CK



Each node shows the sample average rank of staging.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
NORMAL-HIE 1	267.000	100.615	-4.800	.000	.000
NORMAL-HIE 2	50.000	56.119	-4.455	.000	.000
HIE 1-HIE 2	283.500	35.889	2.884	.998	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (1-sided tests) are displayed. The significance level is .05.

Figure 8: CKMB

The ROC curves for determining the cut off values for determining the sensitivity and specificities for the test parameters are as follows. Figure 9, 10, 11, 12 are the ROC curves for LDH, CK, CK-MB, UA levels

respectively for differentiating between Normal and HIE subjects. Figure 13, 14,15 are the ROC curves for CK, CK-MB, UA levels respectively for differentiating between HIE 1 and HIE 2 subjects.

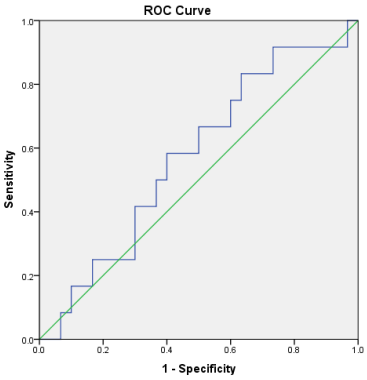


Figure 9

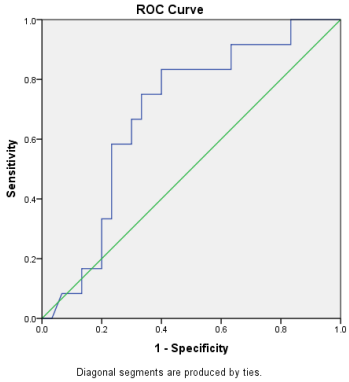


Figure10

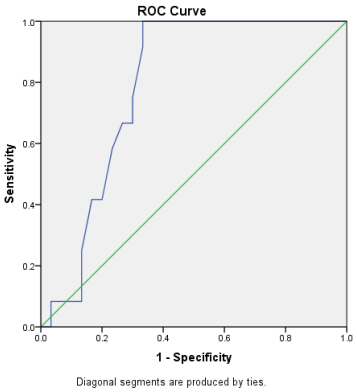


Figure 11

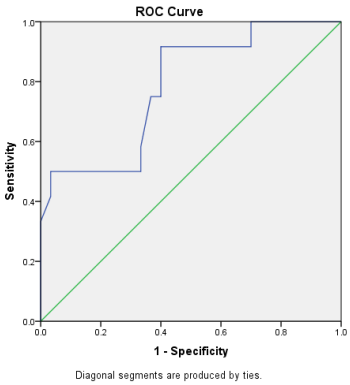


Figure 12

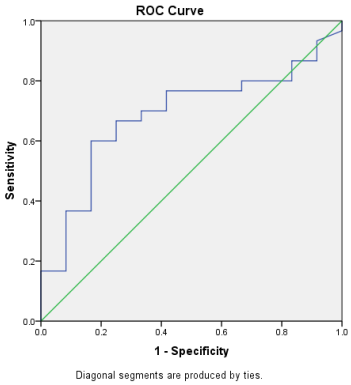


Figure 13

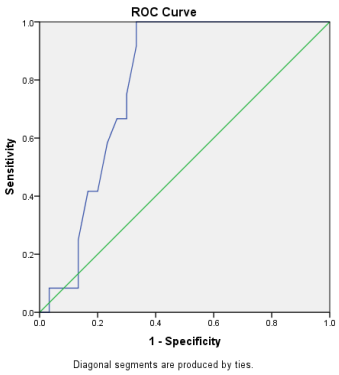


Figure 14

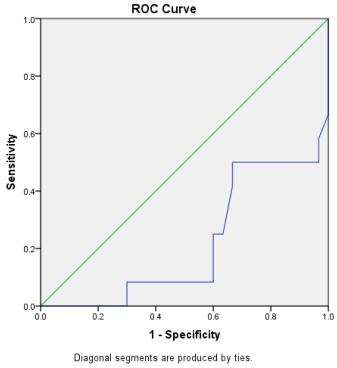


Figure 15

IV. Discussions

Perinatal asphyxia is characterized by progressive damage to all sytems, particularly the nervous system.Lactate dehydrogenase (LDH) an enzyme which catalyses reversibly conversion of pyruvate to lactate during anaerobic glycolysis found extensively in the blood cells, brain cells, heart, mucles and other body tissues . Damage to these cells release large quantities of LDH into plasma. Creatine kinase (CK) catalyses the conversion of creatine to phosphocreatine reversibly using ATP/ADP. In tissue cells that consume ATP rapidly, like brain, retina, etc phosphocreatine acts as an energy reservoir for generation of ATP very fast. In case of damage of these tissues, these enzyme levels in plasma increases.Uric acid (UA) is a metabolic breakdown product of purine nucleotides. It is chemically associated with medical conditions involving extensive tissue damage.

In their study, Fernandez et al [7] and Adhikari et al.[8] have shown that there is significant Increase in CK-BB according to the stages of HIE. But Sweet et al [9] concluded that CK-BB cannot be a predictive marker of brain injury in perinatal asphyxia. Serder Beken eta al[10] demonstrated that the results were conclusive and significant statistically only if LDH, CK and UA were used simultaneously to determine the stages of HIE, as these parameters have a predictive value if measured and interpreted together.

In our study, we found that LDH, CK, CK-MB and UA levels taken within 12 to 24 hours individually give statistically significant results in order to predict HIE in new born infants with birth asphyxia (we did not have any subject with HIE 3 as they were critically ill and hence blood collection was problematic), while UA is also useful in determining and staging the disease as it progresses from HIE 1to HIE 2 with P=0.006. However, UA has a negative correlation with increase in the stage of HIE from 1 to 2. We could not, however investigate as to why there occurred a decrease in level of UA from HIE 1 to HIE 2 due to the short duration and limited contact with study subjects. It is to be noted that the SD of the mean values in most of these parameters show very high ranges. The cause of this can be attributed to the outliers, which may have been subjects with other concomitant causes of the increased values of the respective parameters.

From our study we can say that although there is a definite correlation of LDH, CK, CK-MB and UA levels within first 24 hours of birth with different stages of HIE, the correlation is only found to be statistically significant in case of UA.

On plotting the ROC curves we found that a value of more than equal to 361 for LDH is 85.7% sensitive and 80% specific, a value more than equal to 387.35 for CK is 78.6% sensitive and 78% specific, a value more than equal to 76.995 for CK-MB is 85.7% sensitive and 80% specific while a value of more than equal to 4.45 for UA is 71.4% sensitive and 70% specific in order to diffentiate HIE subjects from normal subjects. To differentiate between HIE 1 & HIE 2, a value of more than equal to 110 for CK-MB is 91.7% sensitive and 67.7% specific, a value of less than equal to 5.335 for UA is 91.7% sensitive and 60% specific, while a value of more than equal to 606.65 for CK is 76.7% sensitive and 60% specific. The levels of LDH did not yield satisfactory cut off values probably due to the insufficiency of study subjects belonging to HIE 2 category, or due to the fact that previous studies confirm that significant rise in LDH levels are observed late, mostly after the lapse of the first 24 hours.

V. Conclusion

Our study reveals that there is statistically significant increase in levels of LDH, CK, CK-MB and UA within first 12 to 24 hours in an asphyxiated new born infant suffering from HIE 1 compared to a normal subject. However, with progress to HIE 2 from HIE 1 only level of UA show a statistically significant change. UA shows an increase in level between normal and HIE 1 subjects, but with progress to HIE 2, the levels decrease, which could not be explained within our study setup. On plotting ROC curves for all the parameters we see that, LDH, CK, CK-MB besides yielding high sensitivity and specificities from progress of a subject to HIE 1 from normal for their respective cut off values, a considerably high sensitivity and specificity is also noted for the cut off values of CK, CK-MB and UA for progress from HIE 1 to HIE 2.

Thus these cut off values maybe used to predict the occurrence and progression of the disease. Testing the levels of these biochemical markers can thus be of great help in determining the onset and progression of the condition in order to start treatment early and have a better outcome.

These results can be better correlated if the duration of the study is increased to incorporate a bigger sample size and the study design is changed to include follow ups of the subjects. We suggest that this study be conducted with a bigger sample size and over a longer duration for more promising results. Samples taken serially on one than one occasion within 24 to 48 hours maybe done for better results of correlations, particularly of LDH.

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