ORIGINAL ARTICLE

STUDY OF MYOCARDIAL INVOLVEMENT AND LACTIC ACID PRODUCTION IN PERINATAL ASPHYXIA

Asutosh P Chauhan[1], Piyush B Tailor[2], Prakash Bhabhor[3], Margeyi M Mehta[1], Rachit M Joshi[4]

[1]Assistant Professor, Dept of Biochemistry, Govt. Medical College and SSG Hospital. Vadodara, Gujarat; [2]Assistant Professor, Dept. of Biochemistry, Govt. Medical College, Surat, Gujarat; [3]Associate Professor, Dept. of Pharmacology, GMERS Medical College, Gotri, Vadodara. Gujarat; [4]Associate Professor, Dept. of Physiology, GMERS Medical College, Dharpur-Patan

Correspondence: Dr. Asutosh Chauhan Email: dr_ashutoshchauhan@yahoo.com

ABSTRACT

Purpose: A Study of newborn infants to evaluate extent of hypoxia from Apgar score and correlate it with biochemistry parameters & to evaluate the presence and extent of cardiac involvement in asphyxiated new born infants along with estimation of the extent of lactic acidosis in asphyxiated new born infants.

Methods: A Study of 52 newborn infants, free from congenital heart diseases were included in the study. After their through clinical examination, they were subdivided into three groups according to their Apgar score. Blood was collected from a stasis free vein in plain bulb. Serum separated after centrifugation was used for estimation of CK-MB and LDH activity.

Results: serum CK-MB levels in group II as compared to group I was significantly raised (P< 0.0001). Similarly the comparison of CK-MB between group II and group III and also between group I and group III was highly significant with a p value of <0.0001. Serum lactate levels were also significantly elevated in cases as compared with controls with a p value of <0.0001.

Conclusions: Our study shows a clear relationship between clinical pattern of asphyxiated newborn infants and alterations of serum cardiac enzymes (CK-MB and LDH) and lactic acid production.

Keywords: perinatal asphyxia, Apgar score, CK-MB, LDH

INTRODUCTION

In spite of major advances in monitoring technology and knowledge of fetal and neonatal pathologies, perinatal asphyxia or, more appropriately, hypoxic-ischemic encephalopathy (HIE), remains a serious condition, causing significant mortality and long-term morbidity [1].

In severe HIE, the mortality rate is as high as 50%. Half of the deaths occur in the first month of life.

If an infant cannot initiate and sustain effective breathing after birth, or if the placenta has malfunctioned before birth, oxygen and carbon dioxide cannot be adequately exchanged resulting in a dangerous drop in the infant's blood oxygen level accompanied by an increase in the carbon dioxide level and accumulation of acid. This combination of events is called perinatal asphyxia (literally meaning suffocating). If not quickly corrected, the heart and especially the brain[2] may be damaged, sometimes irreparably.

Perinatal asphyxia occurs in both premature and term infants. In mild asphyxial episode, chances of permanent injury to the brain and long-term disability are not known. However, if the asphyxial episode is severe, an infant may die, or survive with life-long neurologic disabilities, including cerebral palsy, mental retardation, vision and hearing impairments and learning disabilities.

If hypoxia is severe enough, peripheral tissues develop oxygen debt leading to anaerobic glycolysis and production of a lactacidosis[3]. The lactic acid diffuses into the blood causing a metabolic acidosis. The acidosis depresses cardiovascular function resulting in ischemia. Ischemia is not only results in hypoxia but also lack of delivery of substrates. The result is hypoxic-ischemic disease.

A common accompaniment to birth asphyxia is myocardial ischemia leading to cardiac dysfunction and systemic hypotension. This leads to decrease perfusion of peripheral tissues and vital organ systems, including brain.To evaluate cardiac involvement in neonates with respiratory distress cardiac enzymes were determined. These data were related to clinical presentation and patient outcome [4]. The objective of this study was to

evaluate the level of hypoxia and correlate it with biochemistry parameters and to evaluate the extent of lactic acidosis in asphyxiated newborns.

OBJECTIVES

1. Evaluate extent of hypoxia from Apgar score and correlates it with biochemistry parameters.
2. To evaluate the presence and extent of cardiac involvement in asphyxiated new born infants.
3. To estimate extent of lactic acidosis in asphyxiated new born infants.

MATERIALS AND METHODS

Present study was undertaken at department of biochemistry, SSG Hospital, Baroda and included 52 cases of perinatal asphyxia. Related clinical examination was done and patients were assessed for their serum CK-MB, LDH and plasma lactate[5]. Approval of ethical committee and informed written consent of the parents of the newborns was taken before commencement of this study.

A Study of 52 newborn infants, free of congenital heart disease were included in the study. After their through clinical examination, they were subdivided into three groups according to their Apgar score[6].

Group I consisted of 29 neonates who had 5 min Apgar scores > 9
Group II comprised of 27 neonates with moderate respiratory distress. They had mild symptoms, a 5 min Apgar score ranging between 6 and 9.
Group III included 25 symptomatic neonates with severe asphyxia with 5 min Apgar scores < 6.

Specimen Collection:

Blood was collected from a stasis free vein in plain bulb. Serum separated after centrifugation was used for estimation of CK-MB and LDH activity.

Blood was also collected in fluoride bulb. Plasma was separated by centrifugation within 30 min. A delay in separation can lead to an increase in lactate values.

A: CK-MB

Method: Immunoinhibition / Mod. IFCC method
Procedure Limitation: The procedure assumes that CK-BB activity in the sample is negligible. If a significant amount of CK-BB activity is present then the CK-BB activity will also be overestimated.

B: LACTATE

Method:PAP method
Principle The concentration of L-Lactate in the sample is determined according to the following reaction.

$$\text{L-Lactate} + O_2 \xrightarrow{\text{Lactate Oxidase}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{purple product} + 4H_2O$$

TOOS = N-ethyl-N-(2- hydroxyl-3-sulphopropyl)m-toluidine

C. LDH (Lactate Dehydrogenase)

Method: Modified IFCC
Principle: Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity in the sample.

RESULTS

Total 52 newborns were studied and divided into groups.

Group I consisted of 29 neonates who had 5 min Apgar scores > 9; Group II comprised of 27 neonates with moderate respiratory distress. They had mild symptoms, a 5 min Apgar score ranging between 6 and 9; and Group III included 25 symptomatic neonates with severe asphyxia with 5 min Apgar scores < 6.

Comparison of the serum MB fraction of Creatine Kinase enzyme, Lactate dehydrogenase enzyme and serum lactate levels between different groups was done and following results were obtained.

Table I: Comparison of Serum CK-MB Levels

Group	Patients	Mean Serum CK-MB (Sd)
Group I (control)	29	11.86 (5.09)
Group II (5 min. Apgar score 6-9)	27	71.66 (38.90)
Group III (5 min. Apgar score < 6)	25	200.84 (96.80)

P < 0.0001 for Group I and group II; P < 0.0001 for Group I and group III; P < 0.0001 for Group II and group III

Above three tables show comparison of serum CK-MB levels of Group I, Group II and Group III. Tables show Group I consisted of 29 normal newborn infants having 5 min. Apgar score 9 or above with mean and standard deviation of 11.86 and 5.09 respectively. Group II consisted of 27 newborn infants with 5 min. Apgar score between 6 and 9 with mean and standard deviation of 71.66 and 38.90 respectively. Group III consist of 25 newborn infants with 5 min Apgar score below 6 with mean and standard deviation of 200.84 and 96.80 respectively.

Table II: Comparison of Serum Lactate Dehydrogenase Levels

Group	Patients	Mean Serum CK-MB (Sd)
Group I (control)	29	278.10 (108.22)
Group II (5 min. Apgar score 6-9)	27	734.92 (326.77)
Group III (5 min. Apgar score < 6)	25	1167.60 (373.90)

P < 0.0001 for Group I and group II; P < 0.0001 for Group I and group III; P < 0.0001 for Group II and group III

Above tables shows comparison of serum LDH levels of Group I, Group II and Group III. Group I which consisted of 29 normal newborn infants having 5 min. Apgar score 9 or above with mean and standard deviation of 278.10 and 108.22 respectively. Group II consist of 27 newborn infants with 5 min. Apgar score between 6 and 9 with mean and standard deviation of 734.92 and 326.77 respectively. Group III consist of 25 newborn infants with 5 min Apgar score below 6 with mean and standard deviation of 1167.60 and 373.90 respectively.

Table III: Comparison of Plasma Lactate Levels

Group	Patients	Mean Serum CK-MB (Sd)
Group I (control)	29	11.05 (4.30)
Group II (5 min. Apgar score 6-9)	27	35.64 (9.23)
Group III (5 min. Apgar score < 6)	25	56.18 (13.36)

P < 0.0001 for Group I and group II; P < 0.0001 for Group I and group III; P < 0.0001 for Group II and group III

Above tables show comparison of plasma lactate levels of Group I, Group II and Group III. Group I which consisted of 29 normal newborn infants having 5 min. Apgar score 9 or above with mean and standard deviation of 11.05 and 4.30 respectively. Group II consist of 27 newborn infants with 5 min Apgar score between 6 and 9 with mean and standard deviation of 35.64 and 9.23 respectively. Group III consist of 25 newborn infants with 5 min Apgar score below 6 with mean and standard deviation of 56.18 and 13.36 respectively.

DISCUSSION

In perinatal asphyxia there is impaired tissue perfusion and oxygenation of the vital organs of the fetus. This causes production of lactic acid from pyruvate by lactate dehydrogenase as there is decreased availability of oxygen for TCA to proceed.

In these asphyxiated neonates, hypoxia is often responsible for myocardial ischemia. Despite preferential myocardial perfusion, hypoxia leads to myocardial damage. If hypoxia is very severe, peripheral tissues develop oxygen debt which leads to lactacidosis, due to anaerobic glycolysis. The resulting metabolic academia depresses the cardiovascular function resulting in ischemia. Ischemia not only results from hypoxia but also the lack of delivery of substrates results in hypoxic-ischemic disease.

As ischemia progresses, Creatine phosphate reserves are used up, adenosine triphosphate levels fall, and cardiac tissue becomes more acidic as lactate and other acidic intermediates of glycolysis accumulate [7]. Up to 15 to 20 minutes after ischemic incident, the tissue will recover if it receives oxygen supply. However, after about 20 minutes of ischemic incident, over 60% of the cellular adenosine triphosphate has been used up and the amount of lactate in wet myocardial tissue is 12 times its normal aerobic level.

In addition, all cellular glycogen has been used. Once all the glycogen and Creatine phosphate reserves have been used, dramatic structural changes occur, indicating irreversible cell damage. This also causes damage to cell membrane cytosolic enzymes (CK-MB and LDH) are released into blood stream.

The extent of myocardial involvement and lactacidosis in asphyxiated new born infants has not been studied in detail. So the aim of the present study was to look at the differences in the extent of myocardial involvement and lactacidosis in mild and severe asphyxiated newborn infants as compared to normal control newborn infants. The parameters examined were: CK-MB, LDH activity and plasma lactate concentrations. And the patients were divided into three groups on the basis of their Apgar scores.

Our data indicates that there is a very high correlation between clinical pattern of asphyxiated newborn infants and alterations of serum cardiac enzymes (CK-MB and LDH) and lactic acid production[8,9]. The mean values of these three parameters for the Group-1, i.e. control samples were 200, 1167 and 56.18 for CK-MB, LDH and lactic acid respectively (Table Ia, IIa and IIIa). These values fall within the normal expected range. But there was a clear alteration in the values of these parameters in the Group-2 patients, which had mild symptoms and an Apgar score between 6 and 9. The mean values for Group-2 patients were 71, 734 and 35 for CK-MB, LDH and lactic acid respectively (Table IIa, IIb and IIc). Statistical analysis by unpaired t-test showed very high significance with p values lower than 0.00005 for all the three parameters. This clearly indicates that even in patients with mild symptoms there is significantly high myocardial involvement along with lacticacidosis.

A comparison between the asymptomatic control Group-1 samples and severely symptomatic Group-3 samples showed even higher myocardial involvement and lacticacidosis. The mean values for Group-3 patients were 11, 278 and 11 for CK-MB, LDH and lactic acid respectively (Table IIIa, IIIb and IIIc). Similar statistical analysis by unpaired t-test showed very high significance with p values lower than 0.0000005 for all the three parameters checked. This result was expected since a very significantly high difference was observed in all three parameters between control and mildly symptomatic patients. This raised a further question as to whether there is any difference in these parameters between mild and severely affected infants.

Comparison between these parameters in mildly affected Group-2 infants and severely affected Group-3 infants showed a very clear pattern. The p values obtained by unpaired t-test were found to be greater than 0.000005 for all the three parameters. These p

values clearly indicate that there is higher involvement of myocardium and also lacticacidosis in patients with severe disease as compared to patients with mild disease.

This data clearly indicates that there myocardial involvement and lacticacidosis in infants with Perinatal asphyxia, which varies according to the extent of the disease[10].

CONCLUSION

Our study shows a clear relationship between clinical pattern of asphyxiated newborn infants and alterations of serum cardiac enzymes (CK-MB and LDH) and lactic acid production.

In normal newborns with 5 min Apgar score > 9 (Group I) serum CK-MB and LDH, and plasma lactate within reference range. These infants had no signs of cardiac disease and lactic acidosis.

As the degree of hypoxia increased the serum enzymes CK-MB and LDH, and lactic acid increased. The degree of enzyme alterations and lactic acid production was minor in Group II with respect to Group III, as a manifestation of mild myocardial involvement and lactic acidosis consequent to moderate hypoxia, whereas Group III infants showed elevated levels of CK-MB, LDH and lactate when compared to Group I and Group II infants. These show greater myocardial involvement in severely asphyxiated infants and increased lactic acid production due to severe hypoxia.

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