Original Research Article

Correlation of Apgar score with serum glucose, calcium and electrolytes on the asphyxiated neonates

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ABSTRACT

Background: Neonatal asphyxia is characterized by discrepancy of oxygen during perinatal period that can lead to severe hypoxic ischaemic organ damages followed by a fatal outcome including neurodegenerative diseases, mental retardation, and epilepsies. According to world health organization, four million neonatal deaths occurred each year due to birth asphyxia. Therefore, our study was designed to evaluate the status of serum glucose, calcium, electrolytes, and their correlation with the fetal risk factors associated with birth asphyxia.
Methods: Neonates diagnosed with birth asphyxia were considered as "cases" while neonates birth either normal or by cesarean with having no abnormality were considered as "control". Demographics and possible risk factors of both the mother and neonate were noted. All asphyxiated neonates and controls were chosen to examine for serum glucose, calcium and electrolytes. Automated analyzers were used to estimate serum glucose, calcium, sodium and potassium.
Results: We found that the mean serum glucose level was significantly lower in the asphyxiated neonates compared with controls, and consequently showed very strong positive correlation with the Apgar score. Furthermore, significant reduction levels were observed in serum calcium and sodium in the asphyxiated neonates, showing a linear correlation with the Apgar score. Moreover, higher serum potassium was detected in the asphyxiated neonates than in controls, showing a negative correlation with the Apgar score.
Conclusions: We validated that the examined biochemicals of asphyxiated neonates was strongly correlated with the Apgar score. Our study reinforces for adequate clinical evaluation and biochemical monitoring for early diagnosis to prevent adverse neurodevelopmental outcome.

Keywords: Apgar score, Birth asphyxia, Correlation, Electrolytes, Serum

INTRODUCTION

Birth asphyxia is a clinical condition or an event in the perinatal period (ranging from 5 months before birth to one month after birth), that is namely severe reduction in oxygen supply in the neonates.[1] This condition most commonly results in a drop of maternal blood pressure or interference with regular blood flow to infant's brain during labor.[2] This can also occur due to the interruption of respiratory efforts, or inadequate ventilation. Birth asphyxia is one of the leading causes of newborn deaths

in developing countries.[3] More than a million of asphyxiated neonates who survive (further) may develop long-lasting health problems including cerebral palsy, mental retardation, and speaking, hearing, visual, and learning disabilities.[4] Though asphyxia is caused due to poor maternal health condition or complications during labor, newborn asphyxia would be treatable if postpartum mother and their newborns receive first-aid healthcare during or after labor.[5] It has been reported that some basic and effective resuscitation can save majority of newborns who develop asphyxia.[6] However, many newborn babies are suffered from asphyxia deprived of receiving adequate resuscitative care in the developing world and because of the limitations in skilled providers who know how to use simple resuscitation methods and equipments.[7]

In the clinical aspects, Apgar score assessment is a widely used method to assess the neonatal health against infant mortality.[8] Virginia Apgar, an American anesthesiologist, developed the score in 1952. The Apgar scale is determined by evaluating on five criteria of a newborn baby on a scale from zero to two, then summing up the five values thus obtained. The resulting Apgar score ranges from zero to 10.[8] The five criteria are summarized using words chosen to form a backronym (**A**ppearance, **P**ulse, **G**rimace, **A**ctivity, and **R**espiration).

In the present study, we have analyzed randomly selected newborns either asphyxiated or healthy, and measured the serum glucose, calcium, sodium, and potassium levels in the asphyxiated neonates, and found correlation with the severity of asphyxia by considering with Apgar score.

METHODS

Determination of blood glucose levels

This study was intensively conducted from October 2010 to September 2011 at Neonatal Intensive Care Unit of pediatric ward of Chattagram Maa-O-Shishu Hospital Medical College, Chittagong, Bangladesh. Blood samples were collected as previously described from both control babies and asphyxiated neonates and serum was harvested by centrifuging the blood at 2000 × g for 10 min.[19] An automated KONELAB 3i analyzer (Thermofisher Scientific, Waltham, MA, USA) with the GOD-POD and Hexokinase method as per the instructions given with the kits (Thermofisher Scientific, Waltham, MA, USA) were used to estimate blood glucose levels.[20] Briefly, serum samples were mixed with the kit's colorimetric substrate and horseradish peroxidase (Thermofisher Scientific, Waltham, MA, USA) and the reaction was initiated by addition of glucose oxidase (Sigma-Aldrich, St. Louis, Missouri, USA). The microplate was incubated at room temperature for 30 minutes. The glucose oxidase reacts with glucose to generate hydrogen peroxide that reacts with the colorimetric substrate in the presence of HRP, and convert the colorless substrate into a pink-colored product. The pink product was read at 560 nm. The intensity of the color is proportional to the level of glucose that was present in the samples. Normal ranges used for the purpose of study were: blood glucose >45 mg/dl.

Determination of serum calcium level

Blood samples from both control babies and asphyxiated neonates were analyzed to estimate the calcium levels in serum. Blood was collected as previously described and serum was harvested by centrifuging the blood at 2000 × g for 10 min. An automated KONELAB 3i analyzer (Thermofisher Scientific, Waltham, MA, USA) with the colorimetric CPC method as described previously was used to measure the calcium level.[19,21] Briefly, serum samples were mixed with the Arsenazo III (Sigma-Aldrich, St. Louis, Missouri, USA) that reacts with calcium to form a bluish-purple colored complex and absorbance was taken at 600 - 660 nm. The intensity of the color is proportional to the calcium level that present in the samples. Normal ranges used for the purpose of study were: serum calcium (7.1 – 11.6 mEq/l).

Determination of serum sodium and potassium level

Blood samples from both control babies and asphyxiated neonates were analyzed. Blood were collected as previously described and serum was harvested by centrifuging the blood at 2000 × g for 10 min. An automated EasyLyte® analyzer (Medica Corporation, Bedford, MA, USA) with the Ion Selective Electrode (ISE) method was used to perform sodium and potassium measurements.[19,22] Normal ranges used for the purpose of this study were: serum sodium (133-146 mEq/l), serum potassium (3.7-5.2 mEq/l).

Statistical analysis

Experimental data were analyzed with Prism version 7.0a (GraphPad Software Inc., La Jolla, CA, USA) and plotted as scatter plots. Mann-Whitney tests (95% CI) were performed in the comparisons analysis. *p* value as <0.05 was considered as statistically significant. A liner regression was carried out in the correlation analysis.

RESULTS

We have randomly selected newborns either asphyxiated (cases) or healthy (control), total 400 neonates from both sexes involved in this study. The demographic profile of the cases and controls are shown in Table 1.

Serum glucose level in asphyxiated neonates

We have compared the blood glucose level both in asphyxiated neonates and control babies. Our data have shown that there was a significant reduction in the glucose level of asphyxiated neonates than that in controls (Table 2). This lowered level of serum glucose

suggests that the asphyxiated neonates may developed hypoglycemia. The Linear regression analysis showed the decrease in the blood glucose level in asphyxiated

neonates positively correlated with Apgar score (Figure 1).

Table 1: The demographic profile of the cases and controls.[a]

	Cases	Controls
Total number	200	200
Mean body weight in kg	2.26±0.34	2.81 ± 0.52
Male/Female	91/109	84/116
No. of normal birth	89	178
No. elective cesarean section	9	17
No. emergency cesarean section	102	5
No. of cases with Apgar score between 4 and 6	86 (43%)	–
No. of cases with Apgar score between 3 and 0	114 (57%)	–
No. of cases with hypoxic ischemic encephalopathy (HIE) (total)	152 (76%)	
No. of normoglycemic mother	200	200
No. of deaths (short-term outcome—in admission period)	31 (15.5%)	0

[a]Demographics of both the mother and neonate were noted and questions regarding possible risk factors were asked from mother and file records.

Table 2: Serum glucose, calcium, sodium, and potassium levels in asphyxiated neonates.[a]

Biochemical parameters	Control (n=200) mean±S.D.	Asphyxiated (n=200) mean±S.D.	P value
Serum glucose	63.93±9.34 (mg/dL)	29.11±6.42 (mg/dL)	<0.0001
Serum calcium	10.38±1.24 (mg/dL)	5.62±0.98 (mg/dL)	<0.0001
Serum sodium	139.8±8.32 (mEq/L)	110.6±8.45 (mEq/L)	<0.0001
Serum potassium	4.29±0.43 (mEq/L)	6.039±0.63 (mEq/L)	<0.0001

[a]Biochemical parameters of serum from both asphyxiated and control neonates were measured. Data were analyzed by the Mann-Whitney test with a 95% confidence interval (CI).
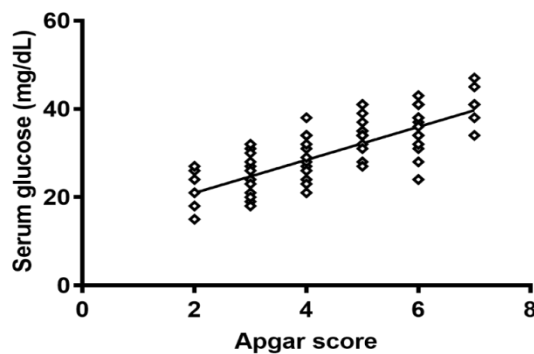

Figure 1: Serum glucose levels showed a strong positive correlation with Apgar score in asphyxiated neonates. A linear regression was performed with 95% CI; (p<0.0001, n=200).

Serum calcium levels in asphyxiated neonates

We measured serum calcium levels in both asphyxiated neonates and controls. We found a decreased level of serum calcium in the asphyxiated neonates when compared with controls (Table 2). This result suggested that asphyxiated neonates were mostly suffered from hypocalcemia whereas controls in the experiment were

under the normal health condition. We next analyzed our data through the linear regression analysis showed that the calcium level in asphyxiated neonates did not show any significant correlation with Apgar score (Figure 2).
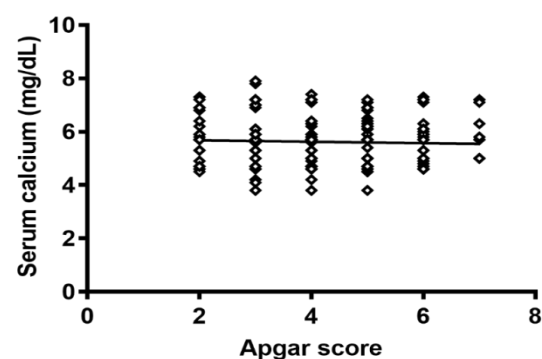

Figure 2: Linear regression analysis of serum calcium levels with Apgar score in asphyxiated neonates (p<0.608, n=200).

Serum sodium levels in asphyxiated neonates

Neonates with asphyxia were found to have reduced levels of serum sodium when compared with the controls

(Table 2), which indicates a risk factor of the asphyxiated neonates that they mostly suffer from hyponatremia. The linear regression analysis showed that the sodium level in asphyxiated neonates was positively correlated with Apgar score (Figure 3).
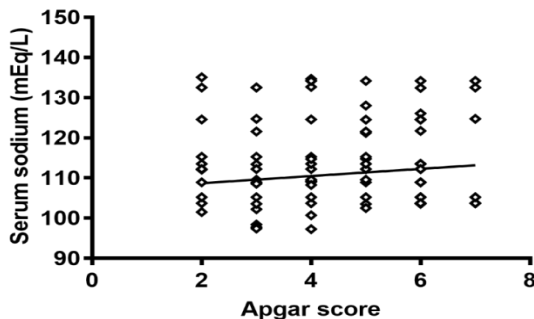

Figure 3: Serum sodium levels showed a positive correlation with Apgar score in asphyxiated neonates. A linear regression was performed with 95% CI; ($p < 0.045$, n=200).

Serum potassium levels in asphyxiated neonates

We have collected blood from both asphyxiated neonates and controls. Serum was harvested and the potassium levels were measured. We have found an increased level of serum potassium in the asphyxiated neonates when compared with controls (Table 2). A negative correlation was observed with Apgar score when data were analyzed by linear regression analysis (Figure 4).
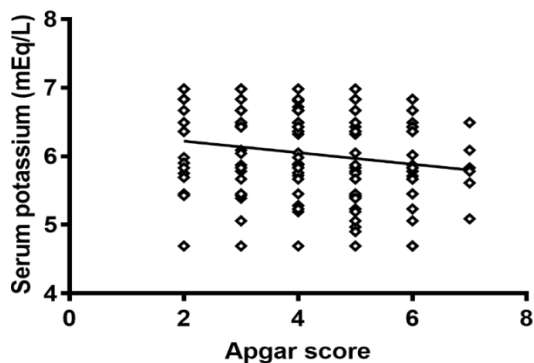

Figure 4: Serum potassium levels showed a negative correlation with Apgar score in asphyxiated neonates. A linear regression was performed ($p < 0.01$, n=200).

DISCUSSION

Birth asphyxia is a common clinical condition during the perinatal period as well as early onset of newborns life which devotes significantly to neonatal mortality and morbidity.[9] Hypoxic ischemic brain injury is considered to be the most severe clinical outcome of perinatal asphyxia.[4] Since, glucose, calcium, sodium and potassium are major biochemical components found in blood mediating most of the biochemical reactions, and thus the deviation from normal levels may be a risk factor for brain injury in asphyxiated neonates as well as further development of numerous consequences including fetal distress, periventricular leukomalacia, developmental delay, mental retardation, seizure disorders, paralysis, and so forth.[10] This study was an attempt to determine the relation and prominence of these biochemical abnormalities in neonates with asphyxia.

In our present study, it was found that there was a significant decrease in the extracellular glucose and this decrease was directly proportional to the degree of severity of birth asphyxia and showed a linear correlation with Apgar score. Our result is consistent with previous results reported by Nadeem et al.[11] The severity of asphyxia may further lead to hypoglycemia and may subsequently cause cellular damage. Sweet et al. had also reported in their study that there was hypoglycemia due to severe asphyxia.[12] This is similar to that observed by Davis et al., in their study that temporary hyperinsulinism contributed to hypoglycemia in the babies with asphyxia.[13] We found that serum calcium levels were significantly dropped in asphyxiated neonates in contrast to controls, which is comparable with several previous by documented reports. Jajoo et al showed that the serum calcium was significantly low in asphyxiated babies, and this lack of calcium (hypocalcemia) consequently developed to hyperphosphatemia that would be identified as possible risk factors in asphyxiated infants.[14] As mentioned previously, hyperphosphatemia has been suggested as a possible etiological factor for hypocalcemia in asphyxiated infants. Higher serum phosphorus levels occur in birth asphyxia due to the endogenous breakdown of glycogen and tissue proteins. Hyperphosphatemia leads to hypocalcemia through increased deposition of calcium in bone, decreased parathyroid action, and increased calcitonin action. The mean serum sodium level also found to be lower in asphyxiated neonates, and apparently showed a positive correlation with the Apgar scores in the asphyxiated neonates being proportional to the degree of asphyxia. The result was in concordance with those of Basu et al.[15] This lowered serum sodium (hyponatremia) which developed perinatally, proportionally contributes to the development of more severe asphyxia if not rectified immediately.[16] The serum potassium level among asphyxiated neonates was significantly higher than that of the controls. This increase in the serum potassium (hyperkaliemia) among the asphyxiated neonates showed a negative correlation with the Apgar score. It may happen due to an immature function of Na^+/K^+ ATPase activity, resulting in an increase of potassium in the serum.[17] Fetal distress, low Apgar scores, and neonatal acidosis or glucose and insulin abnormality have been reported to be significantly associated with the high incidence of potassium in blood.[18]

CONCLUSION

We validated that the predominant biochemical in a neonate is strongly correlated with the Apgar score and

further supporting that increase or decrease in studied parameters correlates with the severity of the degree of birth asphyxia. Our studies reinforce that asphyxiated neonates with abnormal levels of blood glucose, calcium or electrolytes require medical intervention immediate after birth. Furthermore, an adequate clinical evaluation and biochemical monitoring are urgently required for early diagnosis, and proper management to prevent adverse neurodevelopmental outcome and to improve long-term prognosis of neonates with asphyxia. Finally, our study has given a unique opportunity to correlate with the previous studies and a better understanding of the neonates with neonatal asphyxia.

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