Original Article

Iran J Pediatr
Mar 2010; Vol 20 (No 1), Pp:35-40

Measurement of the Urinary Lactate/Creatinine Ratio for Early Diagnosis of the Hypoxic–Ischemic Encephalopathy in Newborns

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Received: Jan *12, 2009*; Final Revision: *Sep 21, 2009*; Accepted: *Nov 14, 2009*

Abstract

Objective: Hypoxic ischemic encephalopathy (HIE) is a major cause of permanent neurological disabilities. Perinatal asphyxia may induce neonatal mortality after birth or neurological impairment among survivors. There are no reliable methods for identifying infants at risk for this disorder.

Methods: We measured the ratio of lactate/creatinine (L/C) in urine by proton nuclear magnetic resonance spectroscopy within 6 and 24 hours after birth in 50 normal infants and 50 infants with asphyxia who developed hypoxic–ischemic encephalopathy. The study was performed from September 2006 to May 2007. For statistical analysis, the SPSS software was used. Group comparisons were performed with chi-square and *t*-test[1,5].

Findings: L/C ratio was 3.3± 2 among asphyxiated neonates in the first six hours after birth which was 11 folds greater than in normal neonates (0.3± 0.08, *P*=0.0001). This ratio decreased to 1.5±0.55 for asphyxiated cases in the first 24 hours after birth, which was 5 folds greater than in control group (*P*=0.0001). Asphyxiated neonates were subdivided into Group A with mild asphyxia and L/C ratio 2.5±0.5; Group B with moderate asphyxia and L/C ratio 4.2±1.5; and Group C with severe asphyxia and L/C ratio 3.4±3.3. The severity of asphyxia correlated with the greater L/C ratio among our cases and was significant (*P*=0.0007). The sensitivity and specificity of L/C ratio in cut off point of 0.48, was 96.1% and 100% respectively.

Conclusion: Measurement of the urinary L/C ratio soon after birth maybe a promising tool to identify asphyxiated neonates and also to predict the severity of asphyxia.

Iranian Journal of Pediatrics, Volume 20 (Number 1), March 2010, Pages: 35-40

Key Words: Hypoxic–Ischemic Encephalopathy; Lactate; Neonate; Asphyxia; Early Diagnosis

Introduction

Perinatal asphyxia is an important cause of neonatal mortality and neurologic disabilities among the infants who survive[1,2]. Newborn infants who sustain an acute intrapartum hypoxic–ischemic insult of sufficient magnitude to result in long-term neurologic sequelae

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invariably have recognizable clinical encephalopathy during the first days of life. They frequently have seizures soon after birth[1,2].

It is important to identify infants at high risk for hypoxic–ischemic encephalopathy in order to provide them proper treatment soon after birth [3,4]. However, most newborns with perinatal asphyxia have unpredictable course; development of hypoxic–ischemic encephalopathy and neurodevelopmental outcome cannot be reliably predicted[1,2,4].

Measurements of hydroxybutyrate dehydrogenase, brain-specific creatine kinase, neuron-specific enolase, lactate dehydrogenase, and interleukin-6, in serum or cerebrospinal fluid may have some value as markers of hypoxic–ischemic encephalopathy[2,5,6].

Severe tissue hypoxia causes the accumulation of intermediary metabolites excreted by the kidneys, notably lactate[7,8], which can be measured readily by proton nuclear magnetic resonance (1H NMR) spectroscopy[9,10]. It was previously reported that increases in urinary lactate excretion could be detected by 1H NMR spectroscopy in newborn infants with perinatal complications[10].

In the present study, we measured urinary lactate and creatinine concentrations within the first 6 and 24 hours after birth and determined sensitivity and specificity of the ratio of urinary lactate to creatinine for the early identification of infants in whom hypoxic–ischemic encephalopathy is likely to develop.

Subjects and Methods

We studied 50 consecutive newborn infants with perinatal asphyxia who were born in our hospitals between October 2006 and June 2007.

Inclusion criteria consisted of gestational age at least 36 weeks and over 2 kg birth weight with perinatal asphyxia and some degree of hypoxic–ischemic encephalopathy. Perinatal asphyxia was defined as the presence of at least three of the following conditions: intrapartum distress as indicated by fetal bradycardia with a heart rate of less than 100 beats per minute, late decelerations, or absence of heart-rate variability, thick meconium-stained amniotic fluid, Apgar score of 6 or less at five minutes; need for resuscitation for more than one minute with positive-pressure ventilation and oxygen immediately after birth and arterial blood pH value of 7.20 or less or a base deficit of at least 14 mmol per liter within the first hour after birth. Hypoxic–ischemic encephalopathy was classified as mild, moderate, or severe on the basis of the staging system described by Santa and Sarnat[11]. This system assesses the infant's level of consciousness, muscle tone, cranial nerves, primitive reflexes, spontaneous motor activity, autonomic function and seizures.

Hypoxic–ischemic encephalopathy was classified as mild if hyperexcitability or hypotonia persisted without seizures for at least 24 hours after birth; as moderate if the infant was lethargic and had hypotonia, weak primitive reflexes, and seizures; and as severe if the infant had frequent seizures, apnea, flaccid weakness, or coma[11].

The exclusion criteria were maternal drug addiction, congenital infections, or perinatal infections, including chorioamnionitis and more than 42 weeks' gestation and development of acute renal failure. The control group consisted of 50 normal, full-term newborns who met the following criteria: no maternal illness, normal results of fetal monitoring, Apgar score of at least 8 at one and five minutes, and a normal course during the first week of life. The infants in both groups were examined daily during the first week after birth by a single examiner who did not know the results of the urinary testing. The study was approved by Ethics Committee of Kurdistan University of Medical Sciences and written informed consent was obtained from the parents of the infants.

Urinary 1H NMR Spectroscopy: Spot urine samples were collected within 6 hours and again 24 hours after birth and were immediately centrifuged. The supernatants were stored at -80°C for later assay. Urinary lactate and creatinine concentrations were measured by high-resolution 1H NMR spectroscopy. The urine samples were prepared for analysis by adding 0.05 ml of deuterium oxide to 0.45 ml of urine contained in a 5-mm NMR tube. The methyl

proton signal of creatinine with a chemical shift set at 3.06 ppm was selected as an internal standard, and the resonance was assigned for lactate and other metabolites. The peak heights for lactate (1.34 ppm) and creatinine (3.06 ppm) were determined, and the ratio of lactate to creatinine was calculated.

For statistical analysis, the SPSS software was used. Group comparisons were performed with chi-square and *t*-test.

Findings

Birth weight, gestational age, and sex were similar among the normal infants, and the infants with asphyxia that developed hypoxic–ischemic encephalopathy (Table 1). All infants with asphyxia developed hypoxic–ischemic encephalopathy. The disease was judged to be mild in 17, moderate in 21, and severe in 12 patients.

Ratio of Lactate to Creatinine in Urine: The mean ratio of L/C in urine within six hours after birth was 3.3±2 in the infants who subsequently developed hypoxic–ischemic encephalopathy- a value that was 11 times as high as that in normal infants (0.3±0.08, $P<0.0001$) (Fig. 1). Urinary L/C ratio of 0.48 or higher had 96.1 percent sensitivity and 100 percent specificity in predicting the development of hypoxic–ischemic encephalopathy. Among the infants who developed hypoxic–ischemic encephalopathy, there was a significant trend for the ratio to increase with the severity of the hypoxic–ischemic encephalopathy in six hours: 2.2±0.5 in the infants with mild encephalopathy, 4.2±1.5 in those with moderate encephalopathy, and 3.4±3.3 in those with severe encephalopathy.

The mean ratio of L/C in urine within 24 hours after birth was 1.5±0.55 in the infants who subsequently developed hypoxic–ischemic encephalopathy - a value that was 5 times as high as the ratio in normal infants (0.3±0.08, $P<0.0001$). Urinary L/C ratio of 0.48 or higher had 98 percent sensitivity and 100 percent specificity in predicting the development of hypoxic–ischemic encephalopathy.

There was a significant trend for the ratio to increase with the severity of the hypoxic–ischemic encephalopathy in 24 hours: 1.1±0.5 in the infants with mild encephalopathy, 1.5±1.5 in those with moderate encephalopathy, and 2±3.3 in those with severe encephalopathy.

Sensitivity and specificity of the urinary L/C ratio at 6 hours after birth was 100 and 96 subsequently, and at 24 hours after birth sensitivity was 100 and specificity 98 (Table 2).

Table 1: Characteristics of 50 normal newborn infants and 50 infants with asphyxia and hypoxic–ischemic encephalopathy

Characteristic	Normal Infants	Infants with asphyxia	*P* value*
Birth weight (g)	3225 ±4.4	3127±350	0.53
Gestational age (wk)	38.8±1.4	37.3±1.3	0.40
Sex (Male/Female)	24/26	27/23	--
Arterial blood gas†	NL	7.09±0.10	--
Base deficit (mmol/liter)	NL	18.5±2.1	--
Apgar score at 1 min (mean)	9	3	--
Urinary L/C‡ ratio at 6 hr	0.3±0.08	3.3±2	<0.001
Urinary L/C ratio at 24 hr	0.3 ±0.08	1.5±0.2	<0.001

* *P* values are for comparisons between the infants with hypoxic–ischemic encephalopathy and normal infants

† Levels of arterial blood gas were measured within the first hour after birth

‡ L/C: Lactate/ Creatinine Ratio

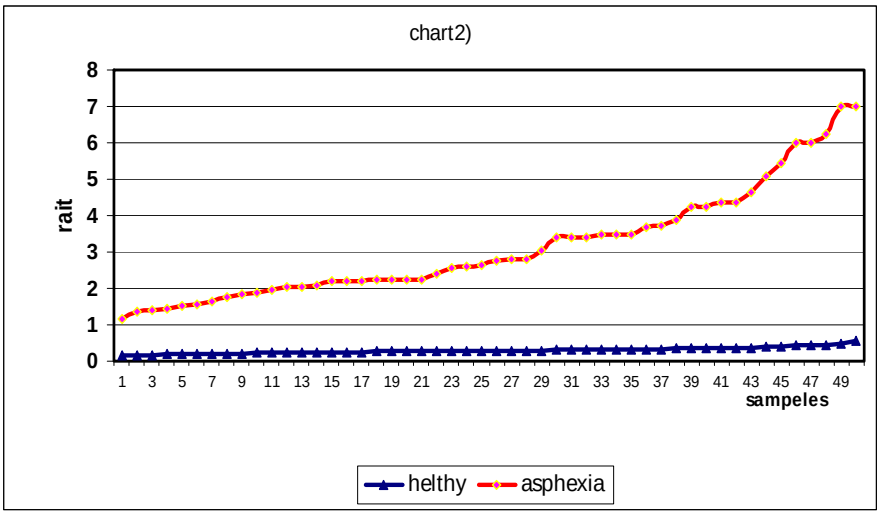

Fig. 1: L/C ratio within six hours after birth in a normal infant, and an infant with asphyxia and hypoxic–ischemic encephalopathy

Discussion

In our study, the newborn infants with asphyxia were examined before hypoxic–ischemic encephalopathy developed. We selected only infants with asphyxia and hypoxic–ischemic encephalopathy. Within six hours after birth, urinary L/C ratios were much higher in the infants that developed hypoxic–ischemic encephalopathy. The ratio also increased as the hypoxic–ischemic encephalopathy worsened. These results suggest that the urinary L/C ratio within six hours after birth is related to the occurrence and degree of hypoxic–ischemic encephalopathy.

Chao-Chang found the same result; it is to be noted that in our study number of infants with asphyxia and hypoxic–ischemic encephalopathy was almost four times more than in their study[7].

The clinical value of this ratio decreases by 24 hours after birth. This suggests that the biochemical derangement detected in the urine after perinatal asphyxia is more pronounced within a few hours after birth than later. The study of Chao-Chang showed in infants with asphyxia the urinary L/C ratio within the first six hours after birth was also significantly related to the neurodevelopmental outcome at one year of age[7].

Table 2: Sensitivity and specifity of urinary lactate/creatinine ratio at 6 hr and24 hr after birth

Urinary lactate/ creatinine ratio	at 6 hr after birth			at 24 hr after birth		
	Patients	Normal	Total	Patients	Normal	Total
Infants with asphyxia	50	2	52	50	1	51
Normal Infants	0	48	48	0	49	49
Total	50	50	100	50	50	100
Sensitivity	96.1			98		
specificity	100			100		
PPV*	96.1			98		
NPV‡	100			100		

* PPV: Positive Predictive Value ‡ NPV: Negative Predictive Value

Kant showed that urinary L/C ratios are much higher in babies with thin meconium, and meconium staining was indicator of perinatal asphyxia of newborns[12].

Our study shows that urinary L/C ratio, determined by 1H NMR within six hours after birth in infants with perinatal asphyxia, can be used to identify most of the infants who will develop hypoxic–ischemic encephalopathy.

The salient abnormality in our study was a marked increase in the urinary L/C ratio within six hours after birth in newborn infants with asphyxia and hypoxic–ischemic encephalopathy.

Lactate is the main end product of anaerobic glucose metabolism. Urinary lactate may result from systemic tissue hypoxia, skeletal-muscle ischemia, or renal injury during asphyxia[2,8,13].

In our study, conventional indicators (Apgar scores, arterial-blood pH, and base deficits) could not be used to predict the development of hypoxic–ischemic encephalopathy, although a multivariate model that incorporated a combination of these markers was somewhat predictive in other studies[1,14,15]. Most studies of perinatal asphyxia have measured biologic markers (brain-specific creatine kinase, hypoxanthine, erythropoietin, and lactate dehydrogenase) in serum or cerebrospinal fluid, but the tests are usually performed several days after birth, when the infants may already have hypoxic–ischemic encephalopathy[2,5,16,17]. These tests may be useful as markers of tissue injury, but they offer little information that can be used to identify newborn infants at high risk for hypoxic–ischemic encephalopathy.

Conditions other than hypoxic–ischemic encephalopathy that may cause high urinary lactate excretion in newborns are acquired diseases (eg, necrotizing enterocolitis)[18] and congenital metabolic disorders (eg, pyruvate dehydrogenasedeficiency, glucose-6-phosphatase–deficient glycogenosis, pyruvate decarboxylase deficiency, propionyl–coenzyme A carboxylase deficiency, and methylmalonic-aciduria)[18,19]. Although metabolic disorders may masquerade as hypoxic–ischemic brain injury in newborn infants, most of these conditions are readily distinguishable from asphyxia. In addition, 1H NMR can also be used to detect these metabolic disorders[18].

As a limitation of the study we could not follow patients after discharge from NICU and compare growth and development in the two groups for determination relationship between urinary L/C ratio and their growth and development.

We suggest further study to compare relationship between urinary L/C ratio at birth and later growth and development.

Conclusion

Our study showed that the urinary lactate/creatinine ratio in newborn infants with asphyxia is useful for predicting the development of hypoxic–ischemic encephalopathy. The L/C ratio may therefore be useful in identifying infants most likely to benefit from intervention.

Acknowledgment

The authors would like to thank the office of vice chancellor for research of Kurdistan University of Medical Sciences for financial support, the university review board for permission to this study.

Conflict of Interest: None

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