

# Measurement of urinary S100B protein levels and lactate/creatinine ratio in early detection of neonatal hypoxic-ischemic encephalopathy

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**Background:** Hypoxic-ischemic encephalopathy (HIE), an important disease in the asphyxiated newborn infants, usually leads to neonatal death and neurological sequelae. There have been no reliable and convenient methods for early identification of HIE. This study was undertaken to investigate the value of urinary S100B protein and lactate/creatinine (L/C) ratio in early diagnosis of neonatal hypoxic-ischemic encephalopathy.

**Methods:** Urinary S100B protein levels and L/C ratio were detected in 58 full-term newborns with HIE on the first, second and third day after birth, and the degree of HIE was assessed within the first seven days after birth. Twenty-five normal full-term neonates were enrolled as controls.

**Results:** The urinary S100B protein levels within the first three days and the L/C ratio on the first day after birth were significantly higher in newborns with HIE than in the controls ( $P < 0.01$ ). There was a positive correlation between urinary S100B protein level on the third day after birth and the L/C ratio on the first day after birth. The two indexes were positively correlated with the clinical grades of HIE ( $P < 0.01$ ). When the S100B protein level was  $0.47 \mu\text{g/L}$  and the urinary L/C ratio was 0.55, their sensitivity and specificity in detecting urinary S100B protein alone on the third day after the birth was 90.4% and 91.9%, respectively. On the first day after birth, the L/C ratio showed the highest sensitivity (91.5%) and specificity (90.3%) in predicting HIE. Detecting the S100B protein level on the third day after birth and the ratio of L/C at the same time on the first

day after birth significantly improved the accuracy in HIE diagnosis, and the sensitivity and specificity under the combined use of the two methods were 98.8% and 97.4%, respectively, which were higher than those from the individual use of the two methods.

**Conclusions:** Based on the clinical manifestations of the asphyxiated full-term newborns, monitoring urinary S100B protein levels and L/C ratio in the first three days after birth is of practical value in improving the accuracy of early diagnosis and clinical grading of HIE.

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**Key words:** newborn;  
hypoxic-ischemic encephalopathy;  
S100B protein;  
lactate;  
creatinine

## Introduction

Hypoxic-ischemic encephalopathy (HIE) caused by perinatal asphyxia is the main cause of neonatal death and neurological sequelae. Thus, early identification of HIE is of great importance in preventing neonatal death and neurological sequelae. However, there have been no reliable and convenient methods for early identification of HIE in the asphyxiated full-term infants. To elucidate the relationship between urinary S100B protein levels and the lactate/creatinine (L/C) ratio in newborn infants with HIE and their diagnostic value as well, we studied 58 neonates with HIE and 25 normal neonates from January 2003 to June 2004.

## Methods

### Subjects

Fifty-eight asphyxiated full-term newborn infants hospitalized at our hospital were enrolled in this

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study. They had been diagnosed as having HIE according to the Criteria<sup>[1]</sup> for Cranial CT Diagnosis and Clinical Grading of Neonatal Hypoxic-Ischemic Encephalopathy set up at the National Congress on HIE held in Hangzhou in October 1996 (23 infants with mild HIE, 22 with moderate HIE, and 13 with severe HIE). Fetal distress occurred in 15 infants and asphyxia in 43 (One minute after birth, 19 newborns showed Apgar score  $\leq 3$  and 24 newborns showed the score from 4 to 7), of whom 11 neonates with asphyxia complicated by fetal distress. In this series, 7 neonates were associated with aspiration pneumonia, 3 with hypoxic-ischemic myocardial damage, 21 with hyperbilirubinemia, 8 with hypoxic-ischemic renal damage. Cranial CT showed disseminated and asymmetrical low density shadows around the ventricles of the brain. There was no clear-cut appearance between the gray and white matter. Of the 58 patients, 24 showed decreased speckled density on CT scan, 23 decreased density in over two sections, and 11 diffuse low-density shadows. Five infants were complicated with subarachnoid hemorrhage. Twenty-five infants with normal physical conditions born in our hospital were enrolled as controls, and their Apgar scores were higher than 8 at 1 and 5 minutes after birth respectively. Mothers of all infants in the control group were healthy. Both groups were full-term newborns and there was no significant difference between the two groups in gestational age, body weight, gender, and mode of delivery (Table 1).

### Research methods

In both groups, 10 ml of urine was collected randomly with disposable urinary collecting bags. The collecting time for the first, second, and third day was  $8 \pm 1.2$ ,  $36 \pm 2.6$ , and  $57 \pm 1.8$  hours, respectively. After the urine was centrifuged at a rate of 3000 cycles/min, the supernatant was collected and kept for later use in a refrigerator at  $-70^\circ\text{C}$ .

Enzyme-linked immunosorbent assay (ELISA) was performed to determine the levels of urinary S100B protein, and the enzyme colorimetric method was used to determine the levels of urine lactate and creatinine. The S100B protein reagent kit used in the present study was bought from Sigma, USA. The urine lactate and creatinine reagent kits were both purchased from Nanjing Jiancheng Biological Co., Ltd., China.

Cranial CT scan was performed for all infants with HIE from the 3rd to the 5th day after birth. The CT images were studied and interpreted by experienced specialists.

### Statistical methods

All the data were expressed as mean  $\pm$  standard deviation (mean $\pm$ SD). SPSS11.0 analytical software was used to analyze the variance of the data between the two groups. Correlation between urinary S100B protein level and the L/C ratio was analyzed. The accuracy and reliability of the two indexes in the prognosis of HIE was assessed.

## Results

### Urinary S100B protein levels and L/C ratio in newborns with HIE of different clinical grades

Urinary S100B concentrations were significantly higher in newborns with HIE than those in the control group ( $P < 0.05$ ). If HIE was subdivided into mild HIE, moderate HIE, and severe HIE, the urinary S100B concentration was significantly higher in newborns with either moderate or severe HIE than in those with mild HIE ( $P < 0.01$ ). No significant difference was seen between newborns with moderate HIE and those with severe HIE ( $P > 0.05$ ). On day 1 after birth, the L/C ratio was significantly higher in newborns with HIE than that in the control group ( $P < 0.01$ ), and was higher in newborns with moderate and severe HIE than in those with mild HIE ( $P < 0.01$ ). On day 2 after birth, except that the L/C ratio was significantly higher in the newborns with severe HIE than that in the control group ( $P < 0.05$ ), no significant difference was observed in the other groups ( $P > 0.05$ ). On day 3 after birth, no significance was found between all the groups ( $P > 0.05$ , Table 2).

### Correlation of urinary S100B protein levels with L/C ratio

Correlation analysis of urinary S100B protein levels, L/C ratio, and clinical HIE grading in the HIE group showed that urinary S100B protein levels were positively correlated with the L/C ratio on the 1st and 2nd day after birth ( $P < 0.01$ ,  $P < 0.05$ ), but not correlated

**Table 1.** Comparison of the HIE and control groups

Group	n	Cesarean section / spontaneous labor	Male/female	Gestational age (wk)	P	Weight (kg)	P
Control	25	6/19	15/10	$38.78 \pm 1.34$		$3.41 \pm 0.47$	
HIE	58	14/44	35/23	$38.52 \pm 1.36$	0.461*	$3.22 \pm 0.36$	0.053*

\*: no significant difference was seen in gestational age and birth weight between the HIE and control groups.

with the ratio on the 3rd day after birth ( $P>0.05$ , Table 3).

### Urinary S100B protein levels, L/C ratio, and the combined use of the two measures in diagnostic evaluation

Further analysis was performed on the accuracy of urinary S100B protein levels and L/C ratio in the diagnosis of HIE. The normal reference ranges were set up according to the values at 95% percentile in the control group. Based on the normal range, the cut-off value of urinary S100B protein level was  $0.47 \mu\text{g/L}$  and the L/C ratio was 0.55, and the sensitivity and specificity were calculated. Urinary S100B protein levels on the third day after birth showed the highest sensitivity and specificity of 90.4% and 91.9% in the diagnosis of HIE respectively, as reported by Gazzolo et al.<sup>[2]</sup> The sensitivity and specificity of L/C ratio in predicting HIE peaked at 91.5% and 90.3% respectively on the first day after birth. If urinary S100B protein level was measured on the third day after birth and L/C ratio was detected on the first day after birth

as well, the sensitivity and specificity in diagnosing HIE would be significantly increased to 98.8% and 97.4%, respectively, and the false positive and negative rates were all decreased. Thus we concluded that the combined detection of L/C ratio on the first day after birth and urinary S100B protein level on the third day after birth is the best method for an accurate diagnosis of HIE, better than the individual detection (Table 4).

## Discussion

### Relationship between S100B levels and HIE

Moore<sup>[3]</sup> first found S100 protein from the extracts of bovine brain tissues in 1965. The protein was named based on its 100% solubility in neutral saturated ammonium sulfate. Further studies in recent years have shown that S100 protein distributes widely in various tissues and it is a calcium-binding EF-hand protein with a low molecular weight of 9-13 kD. S100 protein family is composed of at least 19 components, and S100B is only a member of the family. S100B protein

**Table 2.** Urinary S100B protein levels and urinary L/C ratio in newborns with HIE of different clinical grades and those in the control group (mean  $\pm$ SD)

Group	n	S100B protein levels			L/C ratio		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	25	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02	0.30 $\pm$ 0.04	0.41 $\pm$ 0.05	0.41 $\pm$ 0.05	0.41 $\pm$ 0.05
P		0.008*	0.004*	<0.001*	0.010*	0.061*	0.229*
Mild HIE	23	0.41 $\pm$ 0.05	0.54 $\pm$ 0.16	0.97 $\pm$ 0.39	1.37 $\pm$ 0.12	0.89 $\pm$ 0.31	0.54 $\pm$ 0.16
P		<0.001#	<0.001#	<0.001#	<0.001#	0.317#	0.339#
Moderate HIE	22	3.63 $\pm$ 1.04	3.63 $\pm$ 1.04	0.97 $\pm$ 0.39	4.72 $\pm$ 0.62	0.45 $\pm$ 0.06	0.45 $\pm$ 0.06
P		0.147 $\Delta$	0.096 $\Delta$	0.633 $\Delta$	0.008 $\Delta$	0.057 $\Delta$	0.081 $\Delta$
Severe HIE	13	4.18 $\pm$ 0.76	3.55 $\pm$ 0.62	6.69 $\pm$ 0.86	8.54 $\pm$ 1.22	1.72 $\pm$ 0.79	0.60 $\pm$ 0.33

\*: the HIE group (58 newborns with HIE) vs the control group; #: mild HIE vs moderate HIE;  $\Delta$ : moderate HIE vs severe HIE.

**Table 3.** Coefficients of urinary S100B protein levels and urinary L/C ratio in the HIE group (n=58)

Urinary L/C ratio	Urinary S100B protein		
	Day 1	Day 2	Day 3
Day 1	0.72*	0.69*	0.82*
Day 2	0.33#	0.44#	0.38#
Day 3	0.28	0.35	0.24

\*:  $P<0.01$ ; #:  $P<0.05$ .

**Table 4.** Diagnostic values of urinary L/C ratio, S100B protein levels, and combined use of the two measures

Method	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	FNP (%)	FPP (%)
Urinary S100B protein, D1	81.1	87.4	77.2	74.1	22.8	25.9
Urinary S100B protein, D2	88.5	90.8	80.7	85.7	19.3	14.3
Urinary S100B protein, D3	96.3	100	90.4	91.9	9.6	8.1
Urinary L/C, D1	94.8	100	91.5	90.3	8.5	9.7
Urinary L/C, D2	84.2	80.6	80.6	80.2	19.4	19.8
Urinary L/C, D3	70.1	76.9	74.1	71.1	25.9	28.9
Urinary S100B protein, D3 plus L/C, D1	97.8	100	98.8	97.4	5.2	2.6

PPV: positive predicting value; NPV: negative predicting value; FNP: false negative rate; FPP: false positive rate.

is a dimer composed of two  $\beta$  subunits, whose convergence is guided by hydrophobic force.<sup>[4]</sup> In mammals, the neuron-specific S100B protein has been found in high concentration and widely distributed in neuroglial cells, microglia, macroglia, astrocytes and oligodendrocytes of the central nervous system and in Schwann cells of the peripheral nervous system (The concentration in cerebral tissues is as high as 96%). When there is damage to the brain tissues, increased S100B protein in cerebrospinal fluid enters the blood flow through the injured blood-cerebral barrier (BBB). That is to say, damage to the nervous system may be accompanied by increased S100B protein concentration in cerebrospinal fluid and blood plasma.<sup>[5-15]</sup> In 2001, Gazzolo et al<sup>[16]</sup> determined blood samples of 25 pre-term newborns (a control group of 14 newborns and 11 newborns with HIE) on day 7 after the birth, and found that S100B protein level was higher in newborns with HIE than that in the control group ( $P<0.01$ ) and that the protein level was positively correlated with the hemorrhagic level ( $r=0.7$ ,  $P<0.01$ ). Because urinary S100B protein is mainly metabolized and eliminated via the proximal renal tubules and its biological half-life is about 1 hour and glomerular filtration rate (GFR) is not related with the half-life of urinary S100B protein, GFR has no bearing on the clearance of urinary S100B protein when it is damaged moderately.<sup>[17]</sup> Based on this finding, Gazzolo et al further assayed the urine samples of 45 newborns with HIE and 60 normal healthy newborns in the control group at different time after birth, and found that urinary S100B protein levels were significantly higher in newborns with HIE than those in the control group.<sup>[18]</sup>

In the present study, at different detection time, urinary S100B protein levels were higher in newborns with HIE than those in the control group, and that urinary S100B protein levels were significantly higher in those with moderate and severe HIE than in those with mild HIE and the control group. The changes of urinary S100B protein levels were basically consistent with the clinical grades, i.e., the more severe the brain damage, the higher the urinary S100B protein level. This indicated that urinary S100B was positively correlated with brain damage. It is possibly because fewer neurons of the newborns with mild HIE suffered from necrosis, thus no significant changes were observed in urinary S100B levels; whereas in newborns with severe HIE, marked increase in urinary S100B level was found due to the heavy involvement of the brain, as reported previously.<sup>[2,16,18]</sup> Therefore, it is feasible that urinary S100B protein level can be used as a sensitive and reliable index for early identification of HIE brain damage as well as for assessing the severity

of the damage.

### Relationship between L/C ratio and HIE

Lactate, a fixed acid, is produced from anaerobic glycolysis. It accumulates in the sequent reperfusion damage and it can be eliminated solely through the kidney. After glomerular filtration, creatinine is no longer reabsorbed by renal tubules. Within the first three days after birth, creatinine is mainly in the form of endogenous creatinine and it is rarely affected by external factors. Thus, creatinine is stable in nature. Part of the acid products of metabolism are eliminated via the placenta, thus the blood lactate concentration is not able to reflect the true anoxic condition of the body. However, the products of metabolism in urine have a cumulative effect, so urinary L/C ratio may reflect the general metabolic conditions of the perinatal hypoxia and reperfusion damage. Huang and others<sup>[4,19]</sup> noted that lactate and creatinine both showed apparent peak values in proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMRS). <sup>1</sup>H-NMRS spectrum analysis was performed to determine the urinary L/C ratio of 96 full-term newborns within the first six hours after birth. The results showed that L/C was significantly higher in the newborns who were identified with HIE later, showing satisfactory sensitivity and specificity in predicting HIE development. Its sensitivity and specificity significantly declined at 48 hours after birth. Researches<sup>[9,20-24]</sup> have shown that the results from the enzyme method were consistent with those from <sup>1</sup>H-NMRS. <sup>1</sup>H-NMRS makes assessment possible in general laboratory.

Urinary L/C ratio in the present study was higher in all newborns with HIE than that in the control group, and there were significant differences in urinary L/C ratio between newborns with severe and moderate HIE and the control group. Apart from significant differences between the newborns with severe HIE and the newborns in the control group, there were no significant differences among other groups ( $P>0.05$ ). The overlapping results in the newborns with HIE might be due to the following factors: (1) the small size of sample; (2) different detection methods; and (3) the actual elimination of lactate and creatinine was not proportional to the actual output,<sup>[24-27]</sup> because the renal blood flow is decreased caused by hypoxia-induced redistribution of organic blood flow, it hence leads to the GFR loss, even oliguria and renal function failure.

### Combined measurement of urinary S100B levels and L/C ratio in predicting the development of HIE

In the present study, urinary S100B level was positively correlated with L/C ratio in the HIE



group within the first 3 days after birth. Combined measurement of urinary L/C ratio 24 hours after birth (6 hours the best) and the urinary S100B level on day 3 after birth significantly improved the specificity and sensitivity in diagnosis of HIE, and decreased the false positive and negative rates. Urinary L/C ratio can correctly reflect the anoxic condition of asphyxiated infants. Asphyxia brings damage to multiple organs. Thus the kidney may suffer from the damage to some extent. Because of the cumulative effect of lactate and the stability of endogenous creatinine, measurement of urinary lactate and creatinine can reflect the renal function earlier than serum creatinine and urea nitrogen. In our study, 8 newborns with HIE complicated with hypoxic-ischemic renal damage such as oliguria, proteinuria and hematuria, their L/C ratio was significantly higher than that of those without renal damage, indicating that the increased urinary L/C ratio may be due to the combined effect of renal and brain damage. Hence we determined S100B protein level, specific to the damage of the nervous system, and found they were positively correlated with renal and brain damage. The correlation indicates that when there are both renal and brain damage, the combined measurement of urinary L/C ratio and urinary S100B protein can not only improve the accuracy in the diagnosis of brain damage, but also monitor the renal function so that renal damage induced by HIE can be identified early. Therefore, the combined use of the two methods has higher specificity and sensitivity, can monitor multiple organ damages, and helps to choose specific indexes to reduce amplified diagnosis of HIE.<sup>[26,27]</sup>

In conclusion, early determination of urinary S100B protein and L/C ratio in asphyxiated full-term newborns, combined with the clinical manifestations of HIE, can early detect HIE and its severity. Compared with other humoral specimens, urine sample is easy to collect. The combined measurement is convenient, non-invasive, with a higher specificity. It requires further research in clinical practice.

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**Contributors:** LL proposed the study and wrote the first draft. ZHY analyzed the data. All authors contributed to the intellectual content. NWX contributed to the interpretation.

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