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RESEARCH ARTICLE

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Change of cystatin C values in preterm infants with asphyxia—From two centers of China

Yang Yang[1] | Yue Wu[1] | Jing-jing Pan[2] | Rui Cheng[1]

[1]Department of Neonates, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, China

[2]Department of Pediatrics, The First Affiliated Hospital, Nanjing Medical University, Nanjing, China

Correspondence
Rui Cheng, Department of Neonates, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.
Emails: yy860507@126.com or chengrui350@163.com

Background: To explore the values of cystatin C (Cys-C) in asphyxial preterm babies as an effective endogenous marker of renal function.

Methods: After birth, preterm infants with 5-minute Apgar score <8 were included into the asphyxia group. Finally, 276 preterm infants born in two neonatal intensive care units were studied (including 78 babies in the asphyxia group and 198 babies in the control group). Blood samples were obtained from peripheral veins on day 1, day 7, and day 28 when routine blood screening tests were performed.

Results: In first day samples, the mean levels of Cys-C were 2.21 (1.49-2.98) mg/L with gestational age (GA) >32, 1.94 (1.37-2.76) mg/L with GA 28-32, and 1.87 (1.49-2.13) mg/L with GA <28 in the asphyxia group. In seventh day samples, the mean levels of Cys-C were 2.35 (1.57-3.26) mg/L with GA>32, 2.07 (1.42-2.90) mg/L with GA 28-32, and 1.69 (1.13-2.04) mg/L with GA <28. In twenty-eighth day samples, the mean levels of Cys-C were 1.92 (1.61-2.13) mg/L with GA>32, 1.79 (1.29-1.84) mg/L with GA 28-32, and 1.66 (1.21-2.10) mg/L GA <28. There were significant differences not only between the asphyxia and control groups, but also between the mild, moderate, and severe asphyxia groups.

Conclusion: Cys-C has a good distinguishability in asphyxial neonates in spite of gestational age or birth weight in the Chinese population. Further studies with large numbers of cases are required to assess whether Cys-C could replace creatinine (Cr) and blood urea nitrogen (BUN) as an endogenous marker of renal function.

KEYWORDS

cystatin C, creatinine, blood urea nitrogen, asphyxia

1 | INTRODUCTION

Asphyxia is a common cause of neonatal death and disability, especially for preterm infants. In neonatal intensive care unit (NICU), despite recent advances in perinatal and neonatal resuscitation and treatment, a considerable number of these infants had asphyxia, and it is associated with acute kidney injury (KI) as an independent risk factor.[1,2] Furthermore, renal function is routinely monitored to ensure safe dosing of medicine and to assess hydration status as well as to observe renal effects of various pathologies.[3] Therefore, early detection of KI induced by asphyxia would contribute to efforts for better neonatal outcome.

The most commonly used endogenous filtration markers in clinical practice are serum creatinine (Cr) value or blood urea nitrogen (BUN) value. However, Cr and BUN concentrations are insensitive to detection of mild to moderate reductions in glomerular filtration rate (GFR).[4] In addition, serum Cr and BUN are dependent on age, gender, and accurate evaluation of normal GFR remains difficult in childhood and adults.[5-7]

Cysteine proteases are proteolytic enzymes involved in many pathological processes and are found in the lysosomes of cells. They are essential in normal cellular metabolism, being fundamental to intracellular protein turnover, degradation of collagen, and cleavage of precursor proteins.[8] Cystatin C (Cys-C), which is synthesized

by the proteases, seems to be a more sensitive parameter for the evaluation of GFR because of not having a marked diurnal rhythm, having a constant production rate, being freely filtrated through the glomerulus, and not being influenced by body muscle mass and the gender.[9-11]

In previous researches, there have been few related studies being reported in newborns, especially in preterm babies. In view of this situation, we aimed to compare the values of Cys-C with traditional index (like Cr and BUN) in asphyxial preterm infants from two centers of China for the first time.

2 | PATIENTS AND METHODS

2.1 | Patients

The study was carried out on consecutive babies, born in the First Affiliated Hospital of Nanjing Medical University and Nanjing Children's Hospital of Nanjing Medical University, between March 2014 and March 2016. The two centers are the largest NICUs with 150/200 neonatal intensive care beds in Jiangsu Province. A written informed consent was given by the parents, and the local ethics committee approved the study. After birth, preterm infants with 5-minute Apgar score <8 were included into the asphyxia group. But congenital anomaly, conjugated hyperbilirubinemia, and inherited metabolic diseases were excluded from this study. Finally, 276 preterm infants were enrolled in the study (including 78 babies in the asphyxia group and 198 babies in the control group).

2.2 | Samples

Blood samples were obtained from peripheral veins on the first day, seventh day and twenty-eighth day of life when routine blood screening tests were performed. Cys-C was determined using a particle-enhanced nephelometric immunoassay.[12] Blood Cr and BUN measurements were determined with an automatic biochemical analyzer (Beckman Coulter, Miami, FL, USA).

2.3 | Statistical methods

All calculations were carried out using SPSS (13.0) for Windows. Results were expressed as mean±SD or median+range, depending on normal distribution. The differences between the two groups were assessed using unpaired *t* test or Chi-square test. A *P*-value <.05 was considered statistically significant.

3 | RESULTS

1. We performed comparison of the biochemical parameters between the asphyxia group and the control group according to different gestational ages (GA). As for the demographic characteristics, there were no significant differences on mother's age, gender, birth weight, and gestational age.

In first day samples, the mean levels of Cr were 78.97±22.01, 65.17±25.78, and 56.90±21.66 μmol/L according to different GAs in the asphyxia group. There were no differences between the asphyxia and control groups. The mean levels of BUN were 5.22±1.05, 5.78±3.21, and 5.40±1.80 mmol/L according to different GAs in the asphyxia group. There were also no differences between the two groups. The mean levels of Cys-C were 2.21 (1.49-2.98), 1.94 (1.37-2.76), and 1.87 (1.49-2.13) mg/L according to different GAs in the asphyxia group. There were significant differences between the asphyxia and control groups.

In seventh day samples, the mean levels of Cr were 52.34±24.18, 51.63±21.77, and 62.63±24.39 μmol/L according to different GAs in the asphyxia group. There were no differences between the two groups. The mean levels of BUN were 5.98±1.32, 5.62±0.98, and 5.05±2.85 mmol/L according to different GAs in the asphyxia group. There was a significant difference between the asphyxia and control groups when GA is more than 32 weeks. The mean levels of Cys-C were 2.35 (1.57-3.26), 2.07 (1.42-2.90), and 1.69 (1.13-2.04) mg/L according to different GAs in the asphyxia group. There were significant differences between the asphyxia and control groups.

In twenty-eighth day samples, the mean levels of Cr were 37.33±11.0, 34.75±12.17, and 30.11±9.19 μmol/L according to different GAs in the asphyxia group. There was a significant difference between the asphyxia and control groups when GA is between 28 and 32 weeks. The mean levels of BUN were 2.30±1.13, 2.02±1.22, and 1.63±0.78 mmol/L according to different GAs in the asphyxia group. There were no significant differences between the two groups. The mean levels of Cys-C were 1.92 (1.61-2.13), 1.79 (1.29-1.84), and 1.66 (1.21-2.10) mg/L according to different GAs in the asphyxia group. There was a significant difference between the asphyxia and control groups only when GA is between 28 and 32 weeks (as shown in Table 1).

2. We performed comparison of the biochemical parameters between the asphyxia group and the control group according to different birth weight. In first day samples, the mean levels of Cr were 69.50±10.60, 65.14±26.94, and 61.50±21.12 μmol/L according to different birth weight in the asphyxia group. There were no differences between the asphyxia and control groups. The mean levels of BUN were 6.37±3.81, 5.19±3.04, and 5.36±3.14 mmol/L according to different birth weight in the asphyxia group. There were also no differences between the two groups. The mean levels of Cys-C were 2.15 (1.28-3.95), 2.07 (1.17-3.26), and 1.93 (1.30-3.49) mg/L according to different birth weight in the asphyxia group. There were significant differences between the asphyxia and control groups.

In seventh day samples, the mean levels of Cr were 58.37±12.33, 58.68±16.27, and 60.54±28.88 μmol/L according to different birth weight in the asphyxia group. There were no differences between the asphyxia and control groups. The mean levels of BUN were 6.24±1.77, 5.55±2.36, and 4.96±2.08 mmol/L according to different birth weight

TABLE 1 Comparison of the biochemical parameters between the asphyxia group and the control group according to different gestational ages

Clinical data	GA subgroups (wks)	Asphyxia group	Control group	*P*-value
Age of mother (y)	>32	29.97±5.84	28.63±5.09	.50
	28-32	31.58±5.61	30.22±7.13	.38
	<28	32.12±5.02	30.29±5.89	.41
Birth weight (g)	>32	1399.30±285.81	1354.37±134.04	.49
	28-32	1200.23±183.63	1249.12±157.94	.36
	<28	1059.50±119.84	1038.61±171.88	.67
Gestational age (wk)	>32	33.32±0.61	33.74±0.28	.64
	28-32	31.57±0.44	30.95±0.52	.35
	<28	27.95±0.33	27.22±0.28	.59
Male/female	>32	6/11	21/47	.53
	28-32	25/41	59/132	.12
	<28	15/26	10/19	.21
Cr, day 1 (μmol/L±SD)	>32	78.97±22.01	70.79±26.63	.44
	28-32	65.17±25.78	60.86±19.30	.35
	<28	56.90±21.66	62.66±24.71	.56
BUN, day 1 (mmol/L±SD)	>32	5.22±1.05	5.61±1.92	.54
	28-32	5.78±3.21	4.89±2.46	.12
	<28	5.40±1.80	4.72±1.73	.61
Cys-C, day 1 (mg/L+Range)	>32	2.21 (1.49-2.98)	1.82 (1.53-3.13)	.03
	28-32	1.94 (1.37-2.76)	1.71 (1.59-2.91)	.04
	<28	1.87 (1.49-2.13)	1.63 (1.74-2.02)	.03
Cr, day 7 (μmol/L±SD)	>32	52.34±24.18	49.51±15.59	.36
	28-32	51.63±21.77	46.61±15.68	.17
	<28	62.63±24.39	58.43±22.02	.23
BUN, day 7 (mmol/L±SD)	>32	5.98±1.32	5.01±1.07	.05
	28-32	5.62±0.98	5.45±1.01	.33
	<28	5.05±2.85	4.73±2.65	.15
Cys-C, day 7 (mg/L+Range)	>32	2.35 (1.57-3.26)	1.85 (1.53-3.22)	.02
	28-32	2.07 (1.42-2.90)	1.79 (1.37-2.82)	.03
	<28	1.69 (1.13-2.04)	1.40 (1.21-1.90)	.05
Cr, day 28 (μmol/L±SD)	>32	37.33±11.0	29.04±8.7	.13
	28-32	34.75±12.17	26.31±7.28	.04
	<28	30.11±9.19	35.42±10.30	.29
BUN, day 28 (mmol/L±SD)	>32	2.30±1.13	1.91±1.50	.72
	28-32	2.02±1.22	1.95±0.99	.84
	<28	1.63±0.78	1.27±0.59	.44
Cys-C, day 28 (mg/L+Range)	>32	1.92 (1.61-2.13)	1.80 (1.68-2.14)	.16
	28-32	1.79 (1.29-1.84)	1.49 (1.38-1.79)	.03
	<28	1.66 (1.21-2.10)	1.31 (1.11-1.50)	.08

in the asphyxia group. There were also no differences between the two groups. The mean levels of Cys-C were 2.13 (1.11-3.89), 2.09 (1.04-2.90), and 1.76 (1.03-3.15) mg/L according to different birth weight in the asphyxia group. There were significant differences between the asphyxia and control groups.

In twenty-eighth day samples, the mean levels of Cr were 39.68±10.17, 36.42±9.89, and 29.09±10.88 μmol/L according to different birth weight in the asphyxia group. There were no differences between the asphyxia and control groups. The mean levels of BUN were 3.11±2.09, 2.26±1.11, and 1.46±0.47 mmol/L according

to different birth weight in the asphyxia group. There were no significant differences between the two groups. The mean levels of Cys-C were 1.97 (1.14-4.05), 1.81 (1.03-3.22), and 1.68 (1.10-2.87) mg/L according to different birth weight in the asphyxia group. There was a significant difference between the asphyxia and control groups only when birth weight is <28 or >32 weeks (as shown in Table 2).

4 | DISCUSSION

A low Apgar score is an independent risk factor for impaired renal function in preterm neonates.[13,14] Asphyxia could cause multiple organ damage including KI. Liu et al.[15] conducted a retrospective study and found incidence of renal damage was about 66% in

TABLE 2 Comparison of the biochemical parameters between the asphyxia group and the control group according to different birth weight

Clinical data	BW subgroups (g)	Asphyxia group	Control group	*P*-value
Age of mother (y)	>1500	29.47±6.07	28.39±5.98	.57
	1000-1500	30.85±6.12	30.22±6.13	.49
	<1000	31.29±5.03	30.72±6.22	.63
Birth weight (g)	>1500	1585.00±129.91	1555.00±125.18	.41
	1000-1500	1248.84±142.57	1277.08±133.67	.52
	<1000	871.81±82.56	900.63±89.42	.31
Gestational age (wk)	>1500	33.84±1.67	33.91±1.32	.59
	1000-1500	29.69±1.92	30.72±2.29	.34
	<1000	27.17±1.70	28.25±1.83	.38
Male/female	>1500	6/12	9/16	.57
	1000-1500	23/44	69/162	.20
	<1000	17/22	12/20	.13
Cr, day 1 (μmol/L±SD)	>1500	69.50±10.60	61.50±11.11	.32
	1000-1500	65.14±26.94	63.11±21.93	.63
	<1000	61.50±21.12	70.88±30.52	.34
BUN, day 1 (mmol/L±SD)	>1500	6.37±3.81	5.09±2.70	.73
	1000-1500	5.19±3.04	4.96±2.35	.57
	<1000	5.36±3.14	5.03±3.07	.82
Cys-C, day 1 (mg/L+Range)	>1500	2.15 (1.28-3.95)	1.93 (1.28-3.09)	.05
	1000-1500	2.07 (1.17-3.26)	1.89 (1.35-3.22)	.05
	<1000	1.93 (1.30-3.49)	1.79 (1.37-3.16)	.04
Cr, day 7 (μmol/L±SD)	>1500	58.37±12.33	50.98±10.79	.10
	1000-1500	58.68±16.27	51.37±14.55	.15
	<1000	60.54±28.88	58.50±24.57	.29
BUN, day 7 (mmol/L±SD)	>1500	6.24±1.77	5.32±1.68	.19
	1000-1500	5.55±2.36	4.98±2.54	.17
	<1000	4.96±2.08	4.27±2.15	.12
Cys-C, day 7 (mg/L+Range)	>1500	2.13 (1.11-3.89)	1.80 (1.02-2.99)	.04
	1000-1500	2.09 (1.04-2.90)	1.86 (1.07-2.91)	.05
	<1000	1.76 (1.03-3.15)	1.44 (0.98-2.67)	.04
Cr, day 28 (μmol/L±SD)	>1500	39.68±10.17	30.46±9.93	.09
	1000-1500	36.42±9.89	33.62±11.52	.25
	<1000	29.09±10.88	31.75±12.07	.42
BUN, day 28 (mmol/L±SD)	>1500	3.11±2.09	3.09±1.78	.37
	1000-1500	2.26±1.11	1.88±1.26	.17
	<1000	1.46±0.47	1.21±0.69	.46
Cys-C, day 28 (mg/L+Range)	>1500	1.97 (1.14-4.05)	1.69 (0.99-3.01)	.05
	1000-1500	1.81 (1.03-3.22)	1.56 (1.10-2.12)	.07
	<1000	1.68 (1.10-2.87)	1.36 (0.87-1.67)	.05

asphyxial newborns of China. Therefore, it is necessary to find a sensitive indicator of renal damage. In adults, Cys-C has been demonstrated as a highly sensitive, accurate, and reliable parameter for glomerular filtration rate (GFR) and it is influenced by even mild or moderate changes in GFR. As a good indicator of maturation in GFR, Cys-C level decreases during the earlier days of the life.[16] Our data also support this opinion; the related values gradually decrease with the increase in days after birth (as shown in Tables 1 and 2).

Cys-C is freely filtered at the glomerulus with no tubular secretion. Andersen et al.[17] evaluated the usefulness of Cys-C and concluded that the sensitivity of serum Cys-C for detecting impaired GFR in the pediatric population is superior to that of plasma Cr and BUN. However, there were not able to conclude on the value of Cys-C as a marker in neonates, especially for premature infants. Reference values of Cys-C have been shown for preterm neonates in only a few studies.[18,19] Finney et al.[18] reported that these reference levels as 1.48 mg/L (0.65-3.37) at 24-28 GW and 1.65 mg/L (0.62-4.42) at 29-36 GW. Bariciak et al.[19] also determined these levels as 1.63 mg/L (1.17-2.24) at 24-28 GW and 1.60 mg/L (1.07-2.17) at 28-32 GW on postnatal days 3. In preterm infants, the Cys-C level varied widely in previous reports. With regard to this, Kandasamy et al. summarized a related review, and found that the levels of Cys-C fluctuated from 1.00 to 2.52 mg/L in different researches.[20] Our data showed the mean levels of Cys-C were 2.21 (1.49-2.98) mg/L in infants with GW>32, 1.94 (1.37-2.76) mg/L in infants with GW 28-32, and 1.87 (1.49-2.13) mg/L in infants with GW <28 at first day of life.

In the past reports, Cys-C values did not show significant differences between the asphyxia and control groups in different age groups.[21] But in our study, Cys-C values showed a good distinguishability between the asphyxia group and the control group in spite of gestational age ($P<.05$). In contrast, there were no significant differences on Cr and BUN. But on postnatal day 28, our results showed no statistical difference between the two groups. This may be partly due to gradual improvement in the renal function after kidney damage induced by asphyxia.

Birth weight is another factor which may influence Cys-C level. In this study we divided preterm babies into three groups according to birth weight. We found that Cys-C level showed significant differences between the asphyxia and control groups on postnatal day 1 and postnatal day 7. These determined values were in accordance with the values in the previous studies, which suggest the values of Cys-C are independent of birth weight. We also summarized the Cys-C level in extreme low birth weight infants for the first time (1.79 (1.37-3.16) on day 1, 1.44 (0.98-2.67) mg/L on day 7, and 1.36 (0.87-1.67 mg/L on day 28).

In conclusion, the level of Cys-C in blood sample has a good distinguishability in asphyxial neonates in spite of gestational age or birth weight in the Chinese population. And there could be an increase process following the restoration of glomerular function. Further studies with large numbers of cases are required to assess whether Cys-C could replace Cr and BUN as an endogenous marker of renal function.

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