

Study of Urinary Uric Acid and Creatinine Ratio as a Marker of Perinatal Asphyxia and Its Correlation with Different Stages of Hypoxic Ischemic Encephalopathy

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Abstract

Background: Birth asphyxia is one of the leading causes of neonatal mortality in India. Hypoxic-ischemic encephalopathy (HIE) is the neurological manifestation of systemic hypoxia in new-born. 20-25% of asphyxiated babies who exhibit severe HIE, die during the new-born period. The most commonly used diagnostic and prognostic index to evaluate asphyxia in neonates is APGAR score but alone it is not useful to ferret out neurological outcome. Now-a-days uses of biomarkers enable the clinicians to screen infants for brain injury. We conducted this study to evaluate the role of UUA (urinary uric acid)/Cr (creatinine), which is an early biomarker, in diagnosing and predicting the outcome in perinatal asphyxia.

Aim: To determine the values of UUA/Cr in new-borns with perinatal asphyxia and its relation with different stages of HIE.

Methods: Spot urine samples were collected from the 100 asphyxiated and 100 healthy neonates within 6-24 h of life for determining uric acid and creatinine by auto analyses.

Results: The value of UUA/Cr were statistically significantly higher in the asphyxiated (case) compared with the control group. UUA/Cr ratios were significantly higher in infants with severe HIE (3.61 ± 0.61) when compared with infants with Powered by Editorial Manager[®] and ProduXion Manager[®] from Aries Systems Corporation moderate HIE (2.95 ± 0.98 ; $P < 0.01$) and those with mild HIE (2.64 ± 0.25 ; $P < 0.001$).

Conclusion: UUA/Cr concentration increase considerably after birth asphyxia and is non-invasive, sensitive, early and cost effective method for assessment of asphyxia and its outcome.

Keywords: Asphyxia; Hypoxic ischemic encephalopathy; Biomarkers; Urinary uric acid to creatinine ratio; Outcome

Introduction

As neonatal intensive care has evolved, focus has shifted from improving mortality alone to an effort to improve both mortality and morbidity. Still in developing countries, the perinatal asphyxia continues being the third cause of the neonatal mortality, which is responsible for 23% with 4 million neonates suffering annually from birth asphyxia [1]. Hypoxic-ischemic encephalopathy (HIE) is the neurological manifestation of systemic hypoxia in new-born. The clinical criteria of Sarnat and Sarnat [2] measure the severity of HIE, classifying the patient in three stages according to level of consciousness, muscle tone, posture, tendinous reflexes, presence or absence of myoclonus and change of autonomic functions. Prevalence of perinatal asphyxia varies from 1 to 6 per 1000 live births while incidence of Hypoxic-ischemic injury is about 0.3 to 2 per 1000 full term infants [3]. According to some researchers, 20-25% of asphyxiated babies who exhibit severe HIE die during the new-born period. Babies who survived after severe HIE, upto 25% have permanent neuropsychological handicaps in the form of learning disabilities, epilepsy, cerebral palsy, with or without associated mental

retardation. Systemic asphyxia that causes HIE may occur prior to delivery (placental abruption, toxemia), during delivery (prolonged labour, difficult delivery, abnormal presentation), or after delivery (sepsis, shock). In India between 250,000 to 350,000 infants die each year due to perinatal asphyxia mostly within the first three days of life. In addition, antepartum and intrapartum asphyxia contributes to as many as 300,000 to 400,000 still-births [4]. A clinician's ability to predict the outcome of neonates with HIE is not straightforward. For justifying the use of certain drugs for managing asphyxiated neonates, early recognition of HIE is important. Routinely APGAR score is used to evaluate asphyxia in neonates. But APGAR score alone is not useful to ferret out neurological outcome, because it is influenced by various factors like immaturity, fetal malformations, maternal medications and infection [5].

A key step in the evolution of neonatal neuroprotection is the identification of biomarkers that enable the clinician-scientist to screen infants for brain injury, monitor progression of disease and assess efficacy of neuroprotective clinical trials. Biomarkers are molecules released by or specific to a particular organ. These can be obtained from the blood, urine, cerebrospinal fluid, or any other bodily fluid. In neonates with brain injury, biomarkers may be able to predict the degree and location of injury shortly after the injury occurs. Currently,

clinicians do not routinely use biomarkers to care for neonates with brain injuries [6].

In new-born with perinatal asphyxia anaerobic glycolysis occurs as a result of hypoxic damage to cerebral oxidative metabolism, so only 2 molecules of Adenosine Triphosphate (ATP) are generated as compared to 32 molecules of ATP during aerobic conditions [7]. Further failure of oxidative phosphorylation and ATP production occurs as a consequence of prolonged hypoxia. Lack of ATP and increased cellular destruction will cause an accumulation of Adenosine Monophosphate (AMP) and Adenosine Diphosphate (ADP), which will then get catabolised to its constituents of adenosine, inosine and hypoxanthine [8,9]. Continuous tissue hypoxia and consequent reperfusion injury will result in hypoxanthine being oxidized to xanthine and uric acid in presence of xanthine oxidase. So uric acid production will be increased and cause it to enter blood from damaged tissues. This uric acid will then get excreted in urine where it can be easily detected [10,11].

This study will throw light on important role of urinary uric acid and creatinine ratio (UUA/Cr), that bedside clinician-scientist may use in resource poor setting in diagnosing perinatal asphyxia and stratify babies according to their severity so that neonates will be protected from exposure to unnecessary, ineffective therapies. We hope to contribute to the awareness, validation, and clinical use of these biomarkers.

Materials and Methods

This prospective study was carried out in the department of Paediatrics of SPMC Medical College in Rajasthan from December 2015 to November 2016. The Ethical Committee of the medical faculty approved it, and written informed consent was taken from parents.

The study included 100 asphyxiated new-borns as the study group and 100 healthy new-borns as the control group. For cases:

Inclusion criteria:

- I. Gestational age \geq 37 weeks.
- II. Appropriate for gestational age.
- III. Intrapartum signs of fetal distress.
- IV. Apgar score of <7 min at one minute of life.
- V. Resuscitation with >1 min of positive pressure ventilation before stable spontaneous respiration.
- VI. Mild, moderate or severe hypoxic ischemic encephalopathy as defined by Sarnat and Sarnat [2].

Exclusion criteria:

- I. Congenital malformations.
- II. Maternal drug addiction.
- III. Neonates born to mothers who would have received magnesium sulphate within 4 hours prior to delivery or opioids (pharmacological depression).
- IV. Hemolytic disease of new-born.
- V. Neonates born to mothers consuming alcohol.
- VI. Neonates born to mothers who are smokers.

VII. New-borns born to mothers on antiepileptics.

The control group includes 100 healthy term new-borns, appropriate for gestational age without signs of perinatal asphyxia as evidenced by normal fetal heart patterns, clear liquor and 1 min Apgar score ≥ 7 .

Method of data collection

- Detailed material history, birth events, Apgar score, sex of the baby and weight of the baby were recorded on the pre tested pre structured proforma. Gestational Age was assessed by new Ballard scoring system.
- Thorough clinical and Neurological examination was done for all the neonates included in the study. The asphyxiated neonates (case group) were monitored for seizures, hypotonia, acidemia and HIE in the immediate neonatal period in the NICU. Grading system used to grade the severity of HIE was SARNAT and SARNAT staging. The cases were also observed for other systemic effects of asphyxia.
- Urine samples were collected from the neonates and sent for urinary uric acid and creatinine analysis. The spot urine samples were collected within 6-24 h of life. The procedure was carried out using sterile urine collection bags, after which urine samples were frozen at -20 until analyses could be carried out. Uric acid and creatinine in single urine sample were determined by auto analyses.

Statistical analysis

Data collected was entered into excel sheet and analysed with help of SPSS 22.0 with help of frequency, mean, standard deviation, diagram and appropriate statistical test.

Results

- The present study revealed significant increase in UA/Cr ratio in early spot urine samples from asphyxiated full term new-borns.
- The study proved positive correlation between the urinary UA/Cr ratio and the severity (grading) of HIE ($P < 0.001$).
- UUA/Cr ratios were significantly higher in infants with severe HIE (3.61 ± 0.61) when compared with infants with moderate HIE (2.95 ± 0.98 ; $P < 0.01$) and those with mild HIE (2.64 ± 0.25 ; $P < 0.001$).
- The values of the UA/Cr ratios in the mild and moderate HIE groups were also statistically significant ($P < 0.01$).

Table 1 state that 100 cases and 100 controls were included in the study. Mean gestational age of cases was 37.1 weeks whereas mean gestational age of controls was 37.3 weeks. Among 100 cases, 67% were males and 33% were females whereas among 100 controls, 65% were males and 35% were females. Mean birth weight was slightly lower among new-borns with asphyxia as compared to new-borns without asphyxia (2.92 ± 0.67 kg vs. 3.06 ± 0.71 kg). Among cases, 61% were born through vaginal delivery while 39% were born through caesarean section, whereas among controls 88% were born through vaginal delivery while 12% were born through caesarean section. Among cases 81% had vertex presentation, 10% had breech presentation and 9% had other presentations, whereas among controls 85% had vertex presentation, 11% had breech presentation and 4% had other presentations.

Demographic variables	Cases	Controls
No. of Newborns	100	100
Gestational age(weeks)	37.1 ± 1.61	37.3 ± 1.82
Male/Female	67/33	65/35
Birth Weight	2.92 ± 0.67	3.06 ± 0.71
No. of vaginal deliveries	61	88
No. of LSCS deliveries	39	12
Vertex presentation	81	85
Breech presentation	10	11
Other presentations	9	4

Table 1: Demographic profile of study (case) group and controls.

Table 2 states that cases had 3 times higher UUA/Cr values as compared with controls and also the difference between these two groups was found to be statistically significant (P=0.0001).

UUA/Cr	Cases (N1=100)	Controls (N2=100)
Range	0.78-4.76	0.45-1.21
Mean ± SD	2.68 ± 1.06	0.79 ± 0.36
Z test	16.883	P value=0.0001

Table 2: Comparison of UUA/Cr among cases and controls (UUA/Cr: Urinary Uric Acid Creatinine Ratio).

Table 3 states that the difference among means of UUA/Cr in different stages of HIE cases was observed to be statistically highly significant, also the correlation between HIE and UUA/Cr was positive correlation (r=0.2).

UUA/Cr* HIE	Cases	Mean	SD
1	21	2.649	0.9858
6	53	2.9526	0.9875
3	26	3.6188	0.6103

Table 3: Association of UUA/Cr to the severity of HIE staging (ANOVA=19.705, P=0.0001; UUA/Cr: Urinary Uric Acid Creatinine Ratio; HIE: Hypoxic Ischemic Encephalopathy).

Table 4 states that the cut off UUA/Cr>1.5 among neonatal asphyxia cases, had 91.95% sensitivity, 94.12% positive predictive value, 61.53% specificity and 53.34% negative predictive value. Therefore, this ratio can be used as a sensitive tool to detect neonatal asphyxia.

UUA/Cr	Sensitivity	Specificity	PPV	NPV
>1.5	91.95%	61.53%	94.12%	53.34%

Table 4: Showing sensitivity, specificity and predictive value of UUA/Cr in prediction of Neonatal asphyxia (UUA/Cr: Urinary Uric Acid

Creatinine Ratio; PPV: Positive Predictive Value; NPV: Negative Predictive Value).

Discussion

Perinatal asphyxia is a common neonatal problem and contributes significantly to neonatal mortality and morbidity. There are various methods to diagnose perinatal asphyxia like cranial tomography, somatosensory evoked potentials and magnetic resonance tomography. But these modalities are not useful in first 24 h of life after birth. The APGAR score has a limited role in predicting the immediate outcome, such as that of HIE and the long-term sequelae.

UUA/Cr appears as an early marker of hypoxic ischemic brain injury. Its concentration was significantly elevated in cases as compared with the healthy controls. The new-borns, who were diagnosed with hypoxic ischemic encephalopathy, a significant association was observed between UUA/Cr and Sarnat's grading of the severity of encephalopathy.

We have studied the value of UUA/Cr in asphyxiated new-borns and healthy term neonates within first 24 h of life. The study proved positive correlation between the urinary UA/Cr ratio and the severity (grading) of HIE (P<0.001). UUA/Cr ratios were significantly higher in infants with severe HIE (3.61 ± 0.61) when compared with infants with moderate HIE (2.95 ± 0.98; P<0.01) and those with mild HIE (2.64 ± 0.25; P<0.001). Our results are in concordance with those of Mahmoud and El Abd [11] who reported Urinary UA/Cr ratios were higher in asphyxiated infants (2.9 ± 0.73) when compared with the controls (0.72 ± 0.35, P<0001).

In study by Basu et al. [12] it was found that urinary UA/Cr ratio was significantly higher in cases than controls (3.1 ± 1.3 vs. 0.96 ± 0.54; p<0.001) which is similar to our study.

Another study by Bader et al. [13] showed that UA/Cr was higher in the asphyxiated group when compared to controls. (2.06±1.12, vs. 0.64±0.48; P<0.001) which is also similar to our study.

Our results are also supported by Chen et al. [14] who suggested that urinary ratio of UA to creatinine was significantly higher in both full term and preterm infants with perinatal asphyxia than in those without perinatal asphyxia.

Kumar et al. [15] conducted a study on 110 neonates comprising 55 cases and 55 controls born in Rajendra Institute of Medical Sciences. Spot urine sample collected within first day of life. A cut-off urinary uric acid to creatinine (UA/UCR) ratio value of >1.14 was taken as the cut-off level. The urinary UA/UCR ratios were found to be higher in asphyxiated infants (2.58 ± 1.09) when compared with those in the controls (0.86 ± 0.17 which also is in favour of our study.

Thus the UUA/Cr allows rapid recognition of asphyxia and assessment of its severity and the potential for short term morbidity of death.

Conclusion

Currently diagnosis of perinatal insults relies on adequate documentation of general medicine and obstetrics factors and on radiological and laboratory assessments. But early identification of infants at highest risk for developing seizures to hypoxic ischemia is critical, so that therapeutic strategies can be facilitated. Many specific biomarkers are being investigated now a day to assess damage after perinatal asphyxia in neonates of which UUA/Cr is non-invasive, sensitive, early and cost effective.

So we conclude that UUA/Cr concentration increase considerably after birth asphyxia, and the increase is associated with severity of HIE with a poorer outcome. Hence, UUA/Cr might have an important role in diagnosing and predicting the outcome of perinatal asphyxia.

However, more investigation and studies are required for better understanding of perinatal asphyxia and its outcome.

Limitations

- It is a single centre study with a small sample size, so result could not be generalized on whole population.
- Because specificity of our study is mere 61%, so chances of getting false negative values are more.
- Many of birth asphyxia patients do not pass urine within 24 h of life.
- In our study, we did not correlate UUA/Cr with other biochemical markers.

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