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Clinical significance of urinary uric acid to creatinine ratio in neonates with perinatal asphyxia

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Abstract
In the modern era various biochemical markers are used to establish the diagnosis of perinatal asphyxia and validate the prognosis. Neuron specific enolase, IL-6, glial fibrillary acidic protein, urinary lactate/ creatinine ratio are various markers used to determine the neurodevelopmental outcome. Estimation of interleukin and enzyme require modernized equipment's, expert manpower, and are expensive. Uric acid and creatinine are metabolites, excreted in the urine, the estimation of which is both time and cost effective. Various studies done in the recent past have proved the utility of uric acid/ creatinine ratio as a marker of neonatal asphyxia. The present study was planned to describe prospectively urinary uric acid to creatinine ratio in perinatal asphyxia and showing increased uric acid and creatinine excretion in early spot urine for identification of perinatal asphyxia.
30 Neonates attending the SNCU, Paediatrics Department, Darbhanga Medical College & Hospital, Laheraisarai, Bihar were selected randomly for the present study. Out of them 15 cases were diagnosed as neonatal asphyxia and the other 15 cases were normal neonates.
Perinatal asphyxia is one of the major causes of neonatal mortality and morbidity. It is an important cause of static development and neurological impairment. So early detection and treatment can significantly reduce morbidity and mortality. From the present study it is concluded that urinary uric acid/ creatinine ratio is significantly high in babies with birth asphyxia. There is significant negative linear correlation between urinary uric acid/ creatinine ratio and Apgar score at 1 minute, 5 minutes and 10 minutes.

Keywords: urine uric acid to creatinine, neonatal asphyxia, newborn, etc

Introduction
In spite of major advances in monitoring technology and knowledge of fetal and perinatal medicine, Perinatal asphyxia is one of the significant causes of mortality and long term morbidity. Data from National Neonatal Perinatal database [1] suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India. A gold standard definition of birth asphyxia does not exist. It is probably better to use the term perinatal asphyxia since asphyxia may occur in utero, at birth or in the postnatal period. WHO [2] has defined perinatal asphyxia as a "failure to initiate and sustain breathing at birth" The National Neonatal Perinatal Database (NNPD), 2000 used a similar definition for perinatal asphyxia. It defined moderate asphyxia as slow gasping breathing or an Apgar score of 4-6 at 1 minute of age [1]. Severe asphyxia was defined as no breathing or an Apgar score of 0-3 at 1 minute of age.
According to WHO estimates, around 3% of approximately 120 million infants born every year in developing countries develop birth asphyxia. It is estimated that some 900,000 of these newborns die each year. In India, between 250,000-350,000 infants die each year due to birth asphyxia [2]. Perinatal asphyxia results in hypoxic injury to various organs including kidneys, lungs and liver but the most serious effects are seen on the central nervous system [1, 3]. Hypoxic ischemic encephalopathy (HIE) refers to the CNS dysfunction associated with perinatal asphyxia. The clinical features include altered consciousness, tone problems, seizure activity, autonomic disturbances, abnormalities of peripheral and brain stem reflexes. HIE is of foremost concern in an asphyxiated neonate because of its potential to cause serious long-term neuromotor sequelae among survivors. A detailed classification of HIE (Stage I, Stage II and Stage III) in term neonates was proposed by Sarnat and Sarnat [4]. A simple and practical classification of HIE by severity of manifestations provided by Levene is recommended for routine use [5]. Hypoxic-ischemic brain damage is a gradually evolving process, which begins during the insult and extends beyond the resuscitation period. Although the initial brain injury (phase of energy failure) is related to hypoxia and ischemia, subsequent reperfusion and generation of free radicals contributes to ongoing injury (delayed phase of neuronal injury).
Despite major advances in monitoring technology and knowledge of fetal and neonatal pathologies, hypoxic-ischemic encephalopathy (HIE) remains a serious condition that causes significant mortality and long-term morbidity. HIE is characterized by clinical and laboratory evidence of acute or subacute brain injury due to asphyxia (ie, hypoxia, acidosis). Most often, the exact timing and underlying cause remain unknown. The American Academy of Pediatrics (AAP) and American College of Obstetrics and Gynecology (ACOG) published guidelines to assist in the diagnosis of severe hypoxic-ischemic encephalopathy (see History) [6].
Brain hypoxia and ischemia due to systemic hypoxemia, reduced cerebral blood flow (CBF), or both are the primary

physiologic processes that lead to hypoxic-ischemic encephalopathy (HIE). The initial compensatory adjustment to an asphyxial event is an increase in CBF due to hypoxia and hypercapnia. This is accompanied by a redistribution of cardiac output to essential organs, including the brain, heart, and adrenal glands. A blood pressure (BP) increase due to increased release of epinephrine further enhances this compensatory response. In adults, CBF is maintained at a constant level despite a wide range in systemic BP. This phenomenon is known as the cerebral auto regulation, which helps maintain cerebral perfusion. The physiologic aspects of CBF auto regulation has been well studied in perinatal and adult experimental animals. In human adults, the BP range at which CBF is maintained is 60-100 mm Hg. Limited data in the human fetus and the newborn infant suggest that CBF is stable over much narrower range of BPs [7]. Some experts have postulated that, in the healthy term newborn, the BP range at which the CBF auto regulation is maintained may be only between 10-20 mm Hg (compared with the 40 mm Hg range in adults noted above). In addition, the auto regulatory zone may also be set at a lower level, about the midpoint of the normal BP range for the fetus and newborn. However, the precise upper and lower limits of the BP values above and below which the CBF auto regulation is lost remain unknown for the human newborn.

In the fetus and newborn suffering from acute asphyxia, after the early compensatory adjustments fail, the CBF can become pressure-passive, at which time brain perfusion depends on systemic BP. As BP falls, CBF falls below critical levels, and the brain injury secondary to diminished blood supply and a lack of sufficient oxygen occurs. This leads to intracellular energy failure. During the early phases of brain injury, brain temperature drops, and local release of neurotransmitters, such as gamma-aminobutyric acid transaminase (GABA), increase. These changes reduce cerebral oxygen demand, transiently minimizing the impact of asphyxia. At the cellular level, neuronal injury in HIE is an evolving process. The magnitude of the final neuronal damage depends on the duration and severity of the initial insult, combined with the effects of reperfusion injury, and apoptosis. At the biochemical level, a large cascade of events follow hypoxic-ischemic injury.

Excitatory amino acid (EAA) receptor overactivation plays a critical role in the pathogenesis of neonatal hypoxia-ischemia. During cerebral hypoxia-ischemia, the uptake of glutamate the major excitatory neurotransmitter of the mammalian brain is impaired. This results in high synaptic levels of glutamate and EAA receptor over activation, including N-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4 isoxazole propionate (AMPA), and kainate receptors. NMDA receptors are permeable to Ca++ and Na+, whereas AMPA and kainate receptors are permeable to Na+. Accumulation of Na+ coupled with the failure of energy dependent enzymes such as Na+/ K+ - ATPase leads to rapid cytotoxic edema and necrotic cell death. Activation of NMDA receptor leads to intracellular Ca++ accumulation and further pathologic cascades activation. EAAs accumulation also contributes to increasing the pace and extent of programmed cell death through secondary Ca++ intake into the nucleus. The pattern of injury seen after hypoxia-ischemia demonstrate regional susceptibility that can be largely explained by the excitatory circuity at this age (putamen, thalamus, perirolandic cerebral cortex). Finally, developing oligodendroglia is uniquely susceptible to hypoxia-ischemia, specifically excitotoxicity and free radical damage. This white matter injury may be the basis for the disruption of long-term learning and memory faculties in infants with hypoxic-ischemic encephalopathy.

Intracellular Ca++ concentration increases following hypoxia-ischemia as a result of (1) NMDA receptor activation, (2) release of Ca++ from intracellular stores (mitochondria and endoplasmic reticulum [ER]), and (3) failure of Ca++ efflux mechanisms. Consequences of increases intracellular Ca++ concentration include activation of phospholipases, endonucleases, proteases, and, in select neurons, nitric oxide synthase (NOS). Activation of phospholipase A2 leads to release of Ca++ from the ER via activation of phospholipase C. Activation of proteases and endonucleases results in cytoskeletal and DNA damage. During the reperfusion period, free radical production increases due to activation of enzymes such as cyclooxygenase, xanthine oxidase, and lipoxygenase. Free radical damage is further exacerbated in the neonate because of immature antioxidant defenses. Free radicals can lead to lipid peroxidation as well as DNA and protein damage and can trigger apoptosis. Finally, free radicals can combine with nitric oxide (NO) to form peroxynitrite a highly toxic oxidant.

NMDA receptor activation results in activation of neuronal NOS vias PSD-95 and results in the early and transient rise in NO concentration observed in the initial phase of hypoxia. Inducible NOS is expressed in response to the marked inflammation secondary to cerebral ischemia and results in a second wave of NO overproduction that can be prolonged for up to 4-7 days after the insult. This excessive NO production plays an important role in the pathophysiology of perinatal hypoxic-ischemic brain injury. NO neurotoxicity depends in large part on rapid reaction with superoxide to form peroxynitrite [8]. This, in turn, leads to peroxynitrite-induced neurotoxicity, including lipid peroxidation, protein nitration and oxidation, mitochondrial damage and remodeling, depletion of antioxidant reserve, and DNA damage.

Inflammatory mediators (cytokines and chemokines) have been implicated in the pathogenesis of hypoxic-ischemic encephalopathy and may represent a final common pathway of brain injury. Animal studies suggest that cytokines, particularly interleukin (IL)-1b contributes to hypoxic-ischemic damage. The exact mechanisms and which inflammatory mediators are involved in this process remains unclear. Following the initial phase of energy failure from the asphyxial injury, cerebral metabolism may recover following reperfusion, only to deteriorate in a secondary energy failure phase. This new phase of neuronal damage, starting at about 6-24 hours after the initial injury, is characterized by mitochondrial dysfunction, and initiation of the apoptotic cascade. This phase has been called the "delayed phase of neuronal injury." The duration of the delayed phase is not precisely known in the human fetus and newborn but appears to increase over the first 24-48 hours and then start to resolve thereafter. In the human infant, the duration of this phase is correlated with adverse neurodevelopmental outcomes at 1 year and 4 years after insult [9].

Therapeutic hypothermia is indicated for infants with moderate-to-severe hypoxic-ischemic encephalopathy

(HIE). Supportive management is also critical to prevent additional injury from seizure activity, poor perfusion, electrolyte imbalance, and abnormal glycemic control. Following initial resuscitation and stabilization, treatment of HIE includes hypothermia therapy for moderate to severe encephalopathy as well as supportive measures focusing on adequate oxygenation, ventilation and perfusion, careful fluid management, avoidance of hypoglycemia and hyperglycemia, and treatment of seizures. Intervention strategies aim to avoid any further brain injury in these infants.

In cases of posterior cranial fossa hematoma, surgical drainage may be lifesaving if no additional pathologies are present. In patients with HIE and suspected neonatal sepsis receiving gentamicin and hypothermia treatment, modified gentamicin dosing regimens are required owing to the reduced clearance of this agent potentially leading to toxicity in these infants from higher gentamicin concentrations during hypothermia therapy.

In the modern era various biochemical markers are used to establish the diagnosis of perinatal asphyxia and validate the prognosis. Neuron specific enolase, IL-6, glial fibrillary acidic protein, urinary lactate/ creatinine ratio are various markers used to determine the neurodevelopmental outcome. Estimation of interleukin and enzyme require modernized equipment's, expert manpower, and are expensive. Uric acid and creatinine are metabolites, excreted in the urine, the estimation of which is both time and cost effective. Various studies done in the recent past have proved the utility of uric acid/ creatinine ratio as a marker of neonatal asphyxia. The present study was planned to describe prospectively urinary uric acid to creatinine ratio in perinatal asphyxia and showing increased uric acid and creatinine excretion in early spot urine for identification of perinatal asphyxia.

Methodology

The method used was Case-Control Study and it was conducted in SNCU, Paediatrics Department of Darbhanga Medical College & Hospital, Laheraisarai, Bihar.Total 30 neonates were selected for the present study. Out of them 15 had perinatal asphyxia and the other 15 neonates were normal.

Uric acid level in urine was measured by auto analyzer by uricase / peroxidase method and creatinine was measured by same instrument by alkaline picrate method. The ratio of uric acid and creatinine in both cases and controls were obtained and compared.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion Criteria: Term Babies admitted to SNCU with APGAR score of 6 or less at 5 minutes of birth and controls were apgar score >/=7 at 5 minutes with no signs of asphyxia

Exclusion Criteria: Babies with congenital malformations, suspected metabolic disease on treatment with diuretics, suffering from anuria and those born to mothers having hypertension, diabetes mellitus, toxemia of pregnancy, receiving general anaesthesia, pethidine, phenobarbitone, and other drugs likely to cause depression, in babies and mother febrile attack within 2 months before delivery were excluded from study.

Results & Discussion

The diagnosis of perinatal asphyxia is always associated with uncertainty and standard guidelines to assess the severity are lacking. (6) Despite the increasing understanding of the mechanisms leading to and resulting from perinatal asphyxia early determination of brain damage following hypoxic-ischemic events still remains one of the hardest problems in neonatal care. (1) Their clinical manifestation, their individual potentials to respond to the asphyxia insult to recover vary among the newborns. Prediction of perinatal asphyxia and their severity is essential for the physicians to plan treatment, prognosticate outcome and counsel parents.

In recent studies, new biochemical parameters are being tried with the hope of using them for the definition and diagnosis of perinatal asphyxia. Neuron specific enolase levels in cerebrospinal fluid (CSF), serum hypoxanthine levels, and urinary UA/Cr ratio of asphyxiated infants are accepted as feasible and non-invasive tests [10].

Of late new biochemical parameters are being evaluated with the hope of using them for the definition and diagnosis of perinatal asphyxia. Currently emphasis is being placed on noninvasive, time and cost effective investigatory modalities. Interleukins, enzymes and imaging techniques require experienced personnel well equipped laboratories and are expensive.

UA/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 neonates, who were diagnosed with hypoxic ischemic encephalopathy, a significant association was observed between UA/Cr and Sarnat's grading of the severity of encephalopathy.

Table 1: Demographic Details

Group of	Group A Asphyxiated Babies	Group B Normal Babies
Gestational age	36 – 37 weeks	36 – 37 weeks
Mean birth weight	2.0 – 3.2 kg	2.2 – 3.5 kg
Sex		
Male	12	10
Females	3	5
Number of vaginal delivery	11	12
Number of LSCS	4	3

Table 2: Apgar score

Time	Apgar score	Group A Asphyxiated Babies	Group B Normal Babies
At 1 min	0 - 3	13	0
	4 - 6	2	0
	≥7	0	15
At 5 min	0 - 3	2	0
	4 - 6	4	0
	≥7	9	15
At 10 min	0 - 3	0	0
	4 - 6	2	0
	≥7	13	15

Table 3: Uric Acid (UA) & Creatinine Score (CA)

Group of	Group A Asphyxiated Babies	Group B Normal Babies
Uric Acid (UA) & Creatinine Score (CA)	0.85 – 4.59	0.32 – 2.18

Bader *et al*. found increased UA/Cr ratios in the urinary samples of 18 term newborns asphyxiated in the first 24 hours of life. They concluded that these ratios showed significant correlation with the asphyxia scores [11]. Akisu, from Turkey, also studied UA/Cr ratios in 27 asphyxiated term infants and reported that this urinary ratio is a simple, reliable method for diagnosing perinatal asphyxia and that it correlates with the clinical severity of HIE [12].

Kumar *et al*. [13] 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 when compared with those in the controls which also is in favour of our study.

Dong Wen Bin *et al*. in his study displayed that neonates who had been suffered from asphyxia have higher level of urinary uric acid to creatinine ratio as compared to the non-asphyxiated neonates. It may be used as an indicator for early assessment of aphyxial severity and also renal injury in post asphyxia neonates [14].

In study by Bahubali *et al*. found that urinary UA/CR ratio was higher in the asphyxiated group compared to controls, they also reported a significant negative correlation between this ratio and the APGAR score [15].

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 [16].

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 [17]. 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 [18-19]. 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 [20].

Brief hypoxia impairs cerebral oxidative metabolism leading to an anaerobic glycolysis to generate ATP. During anaerobic glycolysis, one molecule of glucose yields only 2 molecules of ATP as opposed to yielding 38 molecules of ATP during aerobic conditions. During prolonged hypoxia, cardiac output falls and cerebral blood flow is compromised. A combined hypoxic-ischemic insult produces failure of oxidative phosphorylation and ATP production, sufficient to cause the cellular damage. Lack of ATP and increase excitotoxin will leads to cellular damage and accumulation of adenosine diphosphate and adenosine monophosphate, which is later catabolized to adenosine, inosine and hypoxanthine. If there is uninterrupted tissue hypoxia and reperfusion injury, hypoxanthine is oxidized to xanthine and uric acid in the presence of xanthine oxidase leading to an increase in uric acid production, which come out in blood from tissues and excreted in urine.

Limitations of our study are methodological in nature. More details on asphyxiated neonates such as duration of labor, duration of resuscitation, time to first breath, etc may throw more light on the utility and limitations of the lab tests. We are also limited by the fact that this is single center study with a relatively small sample size. Multicentric studies with more sample size will allow us to utilize more variables in multivariate analysis and further improve upon the predictive value of this marker.

Conclusion

Perinatal asphyxia is one of the major causes of neonatal mortality and morbidity. It is an important cause of static development and neurological impairment. So early detection and treatment can significantly reduce morbidity and mortality. From the present study it is concluded that urinary uric acid/ creatinine ratio is significantly high in neonates with perinatal asphyxia. There is significant negative linear correlation between urinary uric acid/ creatinine ratio and Apgar score at 1 minute, 5 minutes and 10 minutes.

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