

Original Research Article

Urinary uric acid or urinary creatinine ratio as a non-invasive marker for perinatal asphyxia

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Received: 29 March 2020

Accepted: 22 April 2020

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ABSTRACT

Background: Perinatal asphyxia is amongst the common problem of neonates and there exists a significant contribution to the neonatal morbidity and mortality. It is observed as a common and a vital cause of the preventable cerebral injury. The prediction of the perinatal asphyxial outcome is very important but dreadful. There is a limited role for APGAR score to predict the immediate outcome, like HIE and the long-term neurological sequelae observational error may happen in APGAR. But the biochemical parameters can truly be relied upon. This study was done to assess urinary uric acid/urinary creatinine ratio (UA/Cr) as a non-invasive marker for perinatal asphyxia and co-relate its absolute value to the degree of the perinatal asphyxia.

Methods: In this prospective case control study conducted in the Pediatrics Department of Shri Ram Murti Smarak Institute of Medical Sciences between Nov 2017 to May 2019, 42 asphyxiated and 42 non-asphyxiated newborns were included. Detailed history and assessment were for all the enrolled newborns. Spot urine samples were sent for the uric acid and creatinine estimation. Results were documented, and statistical analysis was performed.

Results: Urinary uric acid to creatinine ratio used as additional non-invasive, early and easy biochemical marker of the birth asphyxia that biochemically supports severity grading and clinical diagnosis of the asphyxia by APGAR score.

Conclusions: The ratio of the urinary uric acid and creatinine enables rapid and early recognition of asphyxial injury and also the evaluation of its severity and potential for short-term morbidity or death.

Keywords: APGAR score, Perinatal asphyxia, Hypoxic ischemic encephalopathy, Urinary uric acid/creatinine ratio

INTRODUCTION

Perinatal asphyxia is the condition in which there is an impaired gas exchange leading to hypercapnea, hypoxemia, and acidosis in the fetus or neonate. Perinatal hypoxia is among the leading causes of the perinatal mortality in the developing countries. Globally hypoxia of newborn (birth-asphyxia) or fetus (stillbirth) is estimated to account for 23.0% of 4 million neonatal deaths and 26.0% of 3.2 million still-births each year.¹ In India, due to birth-asphyxia, between 2.5 to 3.5 lakhs

infants die each year, mostly within first 3 days of their life. Perinatal asphyxia contributes to almost 20.0% of the neonatal deaths in India as per the data by National Neonatal Perinatal database (NNPD).² Ninety percent of asphyxia affront happen in intrapartum or antepartum periods as the consequence of placental deficiency bringing about a failure to give oxygen and expel carbon dioxide and hydrogen particles from the baby. The staying 10.0% are baby blues typically auxiliary to pneumonic, cardiovascular, or neurologic insufficiency.³ APGAR score is most commonly used as diagnostic and

the prognostic indicator of birth asphyxia, the 1 minute APGAR score shows the need for immediate resuscitation and offsets the 1st golden minute resuscitation. The change in the score between 1 and 5 minutes is the useful index for effectiveness of the resuscitative efforts. But APGAR alone is not useful to ferret out neurologic outcome, which is affected by factors like immaturity, maternal medications, foetal malformations, and infection.⁴ Accurate appraisal of late neurological consequences has failed by performing policies such as foetal heart monitoring, Apgar score. While analysis of hypoxanthine, xanthine, and some inflammatory cytokines are costly, time consuming, and not available routinely for clinical care.⁵

According to the study conducted in Thailand, inappropriate antenatal care, the vacuum extraction, male gender, prolapsed cord and one and five-minute low APGAR scores, were the significant risk factors to the hypoxic ischemic encephalopathy (HIE).⁶

We set out to assess the urinary ratio (UA/Cr) in relation to the APGAR score and the urinary uric acid/urinary creatinine ratio (UA/Cr) as a non-invasive marker for the perinatal asphyxia and co relate its absolute value to degree of the perinatal asphyxia.

METHODS

It was an analytical study carried out in the Pediatrics Department of Shri Ram Murti Smarak Institute of Medical Sciences on term babies. All the term babies (37-41 weeks) born in the hospital or outside and who were admitted in NICU of the hospital with birth asphyxia, who had no other major illnesses were included in the study during the period from November 2017 to May 2019. Study population includes neonates from birth up to 72 hours in the tertiary care hospital. Cases which were fulfilling criteria like term babies admitted in NICU with APGAR score 6 or less at 5 minutes of birth according to NNPD network were included in the study. Similarly in the control group newborn after uncomplicated pregnancy and Apgar score of 7 or more at 5 minutes and no sign of asphyxia were included.

The babies born in tertiary care hospital with features of birth asphyxia and with congenital malformation, suspected metabolic diseases on treatment with diuretics, suffering from anuria, born to mothers having diabetes mellitus, hypertension, toxemia of pregnancy, or received general anaesthesia, phenobarbitone, pethidine or other drugs during delivery causing depression in neonates or mothers having history of febrile attacks within 2 weeks before delivery or preterm and post term babies or those who have given negative consent were excluded from the study. Similarly new born babies with APGAR score 7 or more at 5 minutes and have given negative consent for enrolment in the study were excluded from the control group.

After approval from the Institutional Ethics Committee (IEC) and written informed consent taken, all newborn of age 0 hours to 72 hours who fulfilled the criteria of inclusion were enrolled in the study.

A total of 59 newborn were screened, 7 were rejected to participate in the study and 10 were not fit according to inclusion criteria and finally 42 patients were found eligible according to the given inclusion criteria. All the neonates were blindly examined by an expert doctor and after that routine clinical and pathological examination were done. Local and English language was preferred to ask the screening questions during the initial screening of neonates with valid identity proof and data were recorded. Study tools includes predesigned proforma for data collection, consent form, APGAR Score, Sarnat and Sarnat staging of HIE stage.

A written consent from family members was taken of all potentially eligible subjects after the purpose of study and risk and benefits of participation was explained, those who did not fulfill the inclusion criteria were excluded. Detailed maternal history, APGAR score, birth events, Sarnat and Sarnat staging, gender of the baby and weight of baby were recorded on predesigned proforma from each subject. Gestational age was evaluated by New Ballard scoring system. Prompt clinical appraisal was made by recording respiratory rate, capillary filling time, heart rate, pulse, temperature.

5ml of the arterial cord blood was collected after delivery from following NCCLS (National Clinical Chemistry Laboratory Standards) guidelines.⁷ Cord arterial blood gas-analysis was done on radiometer blood gas analyzer. The spot samples of urine were collected within 24 to 72 hours of life in the infants using the sterile disposable pediatric urine collection bag (200ml capacity) attached to perineum (Romsons Company India). Since only the standard urine-bag was available, it was used for all the newborn infants, irrespective of birth-weight.⁸ Urinary uric acid was measured by the modified Uricase method and urinary creatinine by modified kinetic Jaffe's reaction on Mindray BS 300 auto analyser.

All bio-chemical parameters were analyzed immediately. The markers of tone, heart rate, reflex activity respiratory efforts, and color was used to establish the APGAR score at 1st and 5th minute of the age (maximum score of 10) for Asphyxia <7, for moderate asphyxia 4-6 and for severe asphyxia 0-3. At 72 hours, staging of Hypoxia Ischemic Encephalopathy (HIE) was done by Sarnat and Sarnat staging excluding EEG.⁹ The research procedure was followed in accordance with the approved ethical standards of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, UP, India Ethics Committee (Human).

The data was collected and entered in MS Excel database. After validation, data was analyzed using the statistical program SPSS, version 23.0. Descriptive analyses for the

inter-group comparison were performed using the unpaired 't' test and chi-square test.

Pearson correlation coefficient to test the strength of the association between the cord blood pH and various other variables was performed. Receiver operating characteristic (ROC) plot was used to determine cut-off values of the various parameters. A p-value of <0.05 was considered as significant.

RESULTS

The present study shows there were 34 (81.0%) male and 8 (19.0%) females in case group, while control consisted of 25 (59.5%) male and 17 (40.5%) female; there was significant association ($p < 0.05$). Also, there was a significant association for APGAR score at 1 and 5 minutes among the cases and the controls (Table 1).

Table 1: Demographic distribution of cases and control.

Variables	Cases (n=42) (%)	Control (n=42) (%)	p value
Male	34 (81%)	25 (60%)	0.032
Female	8 (19%)	17 (41%)	
Apgar score at 1st min.	3.76±0.85	7.69±0.60	<0.001
Apgar score at 5th min.	4.90±0.66	8.69±0.60	<0.001
Gestational Age (weeks)	37.48±0.67	37.45±0.74	0.878
First cried time after birth (Seconds)	101.00±127.61	0.00±0.00	<0.001

Table 2: Urinary uric acid and creatinine analysis of study case and control.

Urinary uric acid and creatinine analysis	Case (n=42)	Control(n=42)	p value
Urinary Uric acid	57.77±50.98	34.26±47.40	0.031
Urinary Creatinine	26.99±33.72	42.20±44.99	0.083
Urinary Uric acid/urinary Creatinine (UA/Cr)	4.33±3.57	0.84±1.22	<0.001

Table 3: APGAR score and urinary uric acid to creatinine ratio.

	Case(n=42)	Control (n=42)	p -value
APGAR score at 5 min	4.90±0.6555	8.69±0.6044	<0.001
Urinary Uric acid /Urinary Creatinine(UA/Cr)	4.327±3.57428	0.84±1.21573	<0.001

The correlation between the components of Urinary uric acid and the creatinine analysis of the studied patients and the control were found to be statistically significant ($p < 0.05$) in Uric acid, and Uric acid/urinary creatinine ratio (UA/Cr); while urinary creatinine was insignificantly associated among the groups ($p > 0.05$) (Table 2).

Table 3 shows the mean of APGAR score at 5th minute and urinary uric acid to creatinine ratio values which are similar in both the groups.

The Systemic examination of Baby Higher Mental Function, Tone, Moro's Reflex, Sucking Reflex, Grasp Reflex, Glabellar tap, Tonic Neck, and Plantar Grasp were found to be statistically significant ($p < 0.05$)

In this study as per Sarnat and Sarnat grading scale 47.6% of babies were in HIE stage I, 40.5% and 11.9% in HIE stage II and III respectively. The correlation between Sarnat and Sarnat grading scale i.e HIE staging and various laboratory parameter studies are shown in Table

4. ROC analysis was used to compare the ability of Uric Acid/Urinary Creatinine Ratio to indicate confirmed perinatal asphyxia (Figure 1).

Out of total 84 studied patients 42 were of perinatal asphyxia and Uric Acid/Urinary Creatinine Ratio as cutoff for perinatal asphyxia was found to be 1.25. The area under the curve (AUC) of Uric Acid/Urinary Creatinine Ratio was 0.862 (95% Confidence Interval 0.766-0.959).

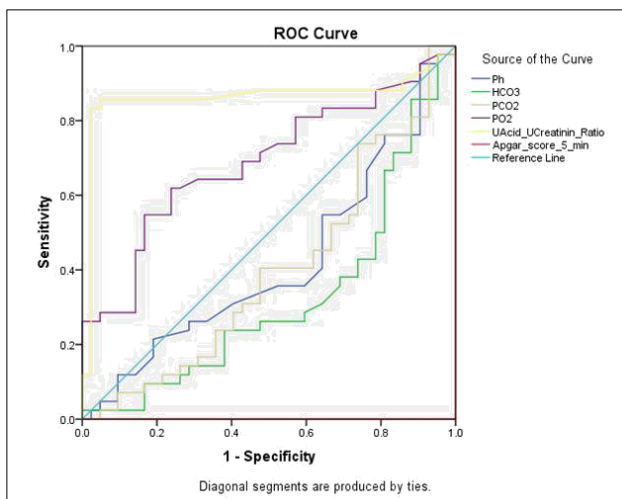
The ROC analysis also showed that the optimal cutoff value Uric Acid/Urinary Creatinine Ratio (>1.25) to indicate confirmed perinatal asphyxia with sensitivity of 85.7%, specificity of 95.2% and accuracy was 86.2%. Apgar score at 5 min showing the cut off for perinatal asphyxia was found 6.5 with sensitivity of 100.0%, specificity of 100.0%. While PO_2 , pH, PCO_2 and HCO_3 accuracy were 68.9%, 41.8%, 39.9% and 32.1% respectively; which are less than the accuracy that the Urinary Uric Acid/Urinary Creatinine Ratio (Table 4, 5 and ROC curve).

Table 4: Mean laboratory parameters on the basis of Sarnat grading scale.

Laboratory parameters	Stage-I (n=20)	Stage-II(n=17)	Stage-III(n=5)	p value
pH	7.40±0.11	7.33±0.10	7.24±0.21	0.037
HCO ₃	20.21±7.05	17.89±5.37	13.76±6.63	0.031
PCO ₂	31.91±10.67	30.16±8.88	30.32±23.55	0.364
PO ₂	111.01±57.93	149.93±135.95	138.60±154.21	0.004
Uric acid	50.06±49.29	63.10±50.66	70.44±64.91	0.134
Urinary Creatinine	25.95±33.25	32.96±38.23	10.80±8.52	0.243
Uric acid/urinary creatinine	4.12±4.20	4.06±3.16	6.04±1.77	<0.001

Table 5: Cutoff, sensitivity, specificity and accuracy of the Ph, HCO₃, PCO₂, PO₂, UA/Cr and APGAR score at 5 min.

	Cutoff point	Sensitivity	Specificity	Accuracy
Ph	7.35	54.8%	35.7%	41.8
HCO ₃	19.9	50.0%	21.4%	32.1%
PCO ₂	32.5	40.5%	42.9%	39.9%
PO ₂	40	88.1%	21.4%	68.9%
UA/Cr.	1.25	85.7%	95.2%	86.2%
Apgar score at 5 th min	6.5	100.0%	100.0%	--

**Figure 1: ROC Curve depicting the sensitivity and specificity of Ph, HCO₃, PCO₂, PO₂, Urinary uric acid/urinary creatinine ratio, APGAR score at 5th minute.**

DISCUSSION

Perinatal asphyxia is the common cause of neonatal morbidity and mortality in developing countries. There are various methods available to diagnose perinatal asphyxia such as magnetic resonance tomography, cranial tomography, and somatosensory evoked potentials. But these modalities are not useful in first 24 hours of life after birth. pH, lactates and base deficits can also be used to diagnose birth asphyxia. Usually, pH, lactates and the base deficits subside with establishment of the respiration, and with other modes of resuscitation. Also,

lactate, pH, base deficit estimations are invasive and require sophisticated instruments and also they are costly, easily available and require expertise. The APGAR score has the limited role in predicting immediate outcome, such as that of HIE and the long-term sequelae.¹

In the present study there were 34 (81.0%) male and 8 (19.0%) females in case group, while control consisted of 25 (59.5%) male and 17 (40.5%) female; there was significant association ($p < 0.05$). Similar results were reported by Sreekrishna Y who depicted that among 50 cases, 60% were males and 40% were females whereas among 50 controls, 60% were males and 40% were females. Krishnana E reported that among the 100 neonates in case group, there were 58 (58%) males and 42 (42%) females.¹⁰ Reddy S also reported significantly higher numbers of males over females in their study.³

In present study Urinary uric acid and creatinine ratios were significantly higher in cases (asphyxiated) than controls (normal newborn), (mean UA/CR ratio in Cases was 4.33 ± 3.57 and in controls was 0.84 ± 1.2 ; $p < 0.001$). Our findings were in accordance with Basu who found that urinary UA/CR ratio was significantly higher in cases than controls (3.1 ± 1.3 vs. 0.96 ± 0.54 ; $p < 0.001$).¹¹ Results are similar to present study. Another study by Bader found that urinary UA/CR ratio was higher in the asphyxiated group compared to controls (2.06 ± 1.12 , versus 0.64 ± 0.48 ; $p < 0.001$).¹² Results correlate with present study. In study by Chen found that urinary UA/CR ratio was higher in the asphyxiated group compared to controls.¹³ This result supports present study. The results of the present study were also in concordance with those of Akisuet who reported elevated

urinary UA/ CR ratio in full term infants with perinatal asphyxia and that the ratio correlates with the severity of HIE.¹⁴ Dong Wen Ben in his study displayed that neonates who had been suffering from asphyxia have the higher level of urinary uric acid to creatinine ratio as compared to the non-asphyxiated neonates.¹⁵ It may be used as an indicator for early assessment of asphyxial severity and also renal injury in post asphyxia neonates.

It was found that in the present study there was the significant difference between Apgar scores of cases than controls ($p < 0.001$). Our findings were similar to the study performed by Krishnana E who reported the APGAR score at 1st minute, 5th minute, 10 minutes was statistically significant between the case and the control group thereby being helpful as an important tool for birth asphyxia diagnosis and its severity.¹⁰ Patel KP also shows the similar results.⁵ Yashwanth K in his study found that urinary UA/Cr ratio was higher significantly in cases than controls (3.1 ± 1.3 versus 0.96 ± 0.54 ; $p < 0.001$) which are similar to my study as shown in table 1.¹⁶ It was also observed that there was statistically significant ($p < 0.05$) difference between Apgar scores of the cases than the controls (3.8 ± 1.4 versus 9.60 ± 0.38 ; $p = 0.02$).

In study conducted by Bahubali it was seen that urinary UA/CR ratio was higher significantly in the asphyxiated group compared to the controls, they also reported the significantly negative correlation between the APGAR score and this ratio.¹⁷ Similar results are given by studies done by Banupriya, Bhongir and Nariman S.^{18,19}

This study also assessed the correlation between urinary Uric Acid/Creatinine (UA/Cr) Ratio to APGAR score at 5th minute, arterial blood gas analysis like pH, $p\text{CO}_2$, $p\text{O}_2$, HCO_3 . ROC curve was made in which area under the curve (AUC) for Urinary Uric Acid/Creatinine (UA/Cr) Ratio was 0.862 and APGAR score at 5th minute was 0.00, which was more than HCO_3 (0.321), $p\text{CO}_2$ (0.399) and $p\text{O}_2$ (0.689) (Table 5).

In the study by Choudhary there was a positive correlation between UA/Cr and the severity of grading of HIE ($p < 0.001$).²⁰ Urinary uric acid to creatinine (UA/Cr) ratio was significantly higher in infants with severe HIE (2.64 ± 0.25). Similar pattern was seen in our study i.e (HIE I 4.12 ± 4.20 , HIE II 4.06 ± 3.16 and HIE III 6.04 ± 1.77 ; $P < 0.001$).

The study done by Kumar found the cut off value by UA/Cr ratio > 1.14 .²¹ Similar study by Bader reported positive predictive value of UA/Cr ratio > 1.2 with sensitivity 74% and specificity 78%.¹² These studies are similar to our study in which 42 newborn were diagnosed with perinatal asphyxia. It was found that UA/Cr ratio was taken as 1.25 to diagnose perinatal asphyxia.

The Area under curve (AUC) of UA/Cr was 0.862 (95% confidence interval 0.766-0.959). The ROC analysis showed the optimal cut off value of UA/Cr (> 1.25) to

indicate perinatal asphyxia with sensitivity 85.7%, specificity of 95.2% and accuracy was 86.2%.

It was also seen that APGAR score at 5th minute with mean value of 6.5 had 100% specificity and sensitivity in diagnosing perinatal asphyxia. It was also found that Urinary Uric Acid/Creatinine (UA/Cr) Ratio and APGAR score at 5th minute had positive correlation. This result was similar to the study done by Basu P in which they also observed the correlation between the APGAR score and Urinary Uric Acid/Creatinine (UA/Cr) Ratio, which they found to be significant ($p < 0.05$).¹¹ However study done by Patel KP and Chen HJ found a negative correlation between APGAR score at 5th minute and Urinary Uric Acid/Creatinine (UA/Cr) Ratio which is not in favor of our study.^{5,13} Kattupalli Y, Babu MS and Srinivas K reported the 94% sensitivity, 96% specificity and accuracy 94.12% values of UA/Cr in prediction of Neo-natal asphyxia in their study.¹⁶

Authors are limited by the fact that this is single center study with a relatively small sample size. In this study, we did not correlate urinary uric acid/ creatinine ratio with other biochemical markers.

This study successfully evaluated the utility of UA/Cr ratio as non invasive, cheap easy, and also at the same time early-biochemical means of the asphyxia diagnosis and to find out whether the APGAR score is still an important tool for birth-asphyxia diagnosis and its severity.

CONCLUSION

Urinary uric acid to the creatinine ratio can be used as additional easy, non invasive, and at the same time early-biochemical marker of birth-asphyxia which biochemically supports clinical diagnosis and the severity grading of the asphyxia by apgar score. Urinary uric acid/ creatinine ratio is significantly high in babies with birth asphyxia and there is significantly negative linear correlation between urinary uric acid/ creatinine ratio and Apgar score at 1 min, 5 min and 10 min among asphyxiated babies.

Recommendations

This study was done with small sample size, so results cannot be generalised, needs further studies with large sample size. Many birth asphyxia babies do not pass urine in the first 24 hrs of life.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the support and help of Dr. (Brig.) P.L. Prasad, Head of the Department of Pediatrics, SRMS-IMS. Author also acknowledge the support and help of Dr. Preeti Lata Rai, Associate Professor of the Department of Pediatrics SRMS-IMS in giving me practical suggestion and guidance in

completion of this work. Authors are highly indebted to Shri Ram Murti Medical College Bareilly especially to Department of Biochemistry for providing me experimental support

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee (Humans) of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, UP, India

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Cite this article as: Siddharth, Rai PL, Prasad PL. Urinary uric acid or urinary creatinine ratio as a non-invasive marker for perinatal asphyxia. *Int J Contemp Pediatr* 2020;7:1378-83.