IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

Open Access
Original Article

Urinary Uric Acid and Creatinine Ratio as a Marker of Perinatal Asphyxia

Kartika Gulati[1], Shree Krishan Vishnoi[1], Sandeep Choudhary[1*], Rakesh Jora[1]

1. Department of Pediatrics, Dr. S N Medical College, Jodhpur, Rajasthan, India

ABSTRACT

Background: Prediction of the outcome of perinatal asphyxia (PA) is important but formidable. Apgar score has a limited role in predicting the outcome. Urinary uric acid and creatinine ratio (UA/Cr) is an early, noninvasive, and cheap biomarker of PA which may predict its morbidity. This study aimed to determine the urinary UA/Cr in neonates with PA, compare it with UA/Cr in non-asphyxiated neonates, and derive the optimum cut-off value of this ratio to label PA.

Methods: This observational cross-sectional study was carried out on 100 term neonates appropriate for gestational age (AGA) with PA (cases) and 100 non-asphyxiated term AGA babies (controls). Urine samples were collected within 24 h of life; moreover, uric acid and creatinine levels were determined by an auto-analyzer.

Results: The mean urinary UA/Cr ratio was significantly higher in the cases, compared to the controls (3.41±0.68 vs. 1.99±0.23) (P<0.0001). The cut-off value of this ratio to label PA was>2.5 with sensitivity, specificity, positive predictive value, and negative predictive value of 98%. Urinary UA/Cr ratio and cord blood pH were significantly correlated with each other (correlation coefficient r=-0.8951, P<0.001). Moreover, the urinary UA/Cr ratio and 5-min Apgar score were also significantly correlated with each other (r=-0.8806, P<0.001).

Conclusion: Urinary UA/Cr ratio is a non-invasive, cheap, and reliable marker for PA with good predictive value in this study.

Keywords: Cord blood pH, Perinatal asphyxia, Urinary UA/Cr ratio

Introduction

Despite improving healthcare and obstetric management, perinatal asphyxia (PA) significantly contributes to newborn mortality and morbidity accounting for 23% of the four million newborn deaths each year globally (1). Cord blood pH, lactate, lactate dehydrogenase (LDH), neutrophil gelatinase-associated lipocalin, neutrophil count, serum and urine elevation of the S100B protein, magnetic resonance imaging of the brain, magnetic resonance spectroscopy of the brain, and electroencephalogram changes have been evaluated as potential biomarkers of PA and specific organ damage by many researchers in recent years. The diagnostic and prognostic values of some of these markers are now established and are a part of the standard management protocol; however, many of these and other biomarkers need continued research to prove their validity. Uninterrupted tissue hypoxia and reperfusion injury lead to the oxidation of hypoxanthine to xanthine and uric acid in the presence of xanthine oxidase causing an increase in uric acid production, which reaches the blood from the tissues and is excreted in the urine (2-4). Therefore, an elevated uric acid and creatinine ratio (urinary UA/Cr ratio) may be a valuable indicator of the severity of tissue hypoxia in patients with intact renal functions. This test offers the advantage of being non-invasive, cheap, and easy to perform and gives early biochemical results. The present study aimed to determine the urinary UA/Cr ratio in neonates with PA and compare it with non-asphyxiated neonates as the primary objective. Secondary objectives of this

* *Corresponding author*: Sandeep Choudhary, Department of Pediatrics, Dr. S N Medical College, Jodhpur, Rajasthan, India. Tel: +9414416405; Email: sandeepbugasara@gmail.com

Please cite this paper as:
Gulati K, Krishan Vishnoi S, Choudhary S, Jora R. Urinary Uric Acid and Creatinine Ratio as a Marker of Perinatal Asphyxia. Iranian Journal of Neonatology. 2022 Jan: 13(1). DOI: 10.22038/IJN.2021.55457.2056

study were to demonstrate the optimum cut-off value of the urinary UA/Cr ratio to label PA and the relationship of this ratio with cord blood pH and Apgar score as a marker of PA.

Methods

This observational cross-sectional study was carried out in the neonatal intensive care unit (NICU) of a tertiary care hospital after receiving the required approval from the Institutional Ethics Review Committee of the institution using the following inclusion and exclusion criteria. This study included 100 intramural term neonates appropriate for gestational age (AGA) with 5-min Apgar scores<7 and cord blood pH<7.00 that were labeled as PA cases (5). Moreover, 100 non-asphyxiated intramural term AGA neonates with 5-min Apgar scores>7 were regarded as controls. The cases and controls were matched for weight and gestational age (GA). On the other hand, neonates with major congenital abnormalities and acute kidney injury (AKI) according to the paediatric RIFLE criteria (6), and those whose parents did not give consent, as well as mothers receiving general anaesthesia/narcotics, were excluded from the study.

The sample size calculated for this study was based on the following formula for the comparison of two means:

$$n = \frac{2(Z_{1-\alpha/2} + [h1] Z_{1-\beta})^2}{Standard\ effect\ size}$$

The sample size was calculated at 98 cases using a power of 80%, confidence interval (CI) of 95%, and standard effect size of 0.4. Eventually, it was rounded off to 100. In total, 134 term AGA neonates with 5-min Apgar score<7 (Cases) were selected, out of whom 26 and 5 neonates cases were excluded as they had cord blood pH >7.0 and AKI, respectively. Moreover, the parents of 3 newborns were not willing to participate; therefore, they were excluded from the study. On the other hand, 104 term AGA neonates with a 5-min Apgar score≥7 were selected as controls. It is worth mentioning that 3 neonates had AKI, and the parents of one newborn did not give consent; accordingly, they were excluded from the study. The effective sample size was 100 neonates in each case and control group (Figure 1).

Baseline maternal, antenatal, intrapartum, and neonatal details were recorded in a predesigned proforma of all included neonates. Resuscitation of neonates was conducted as per neonatal resuscitation protocol of the National Neonatology Forum (7). Apgar scores at 1, 5, and 10 min were noted in both cases and controls .

GA was calculated using the New Ballard scoring system. A thorough clinical/neurological examination was performed; moreover, and Sarnat and Sarnat staging (1976) was used to grade hypoxic-ischaemic encephalopathy (HIE) severity (8). Complete blood count, C-reactive protein, blood sugar, calcium, urea, creatinine,

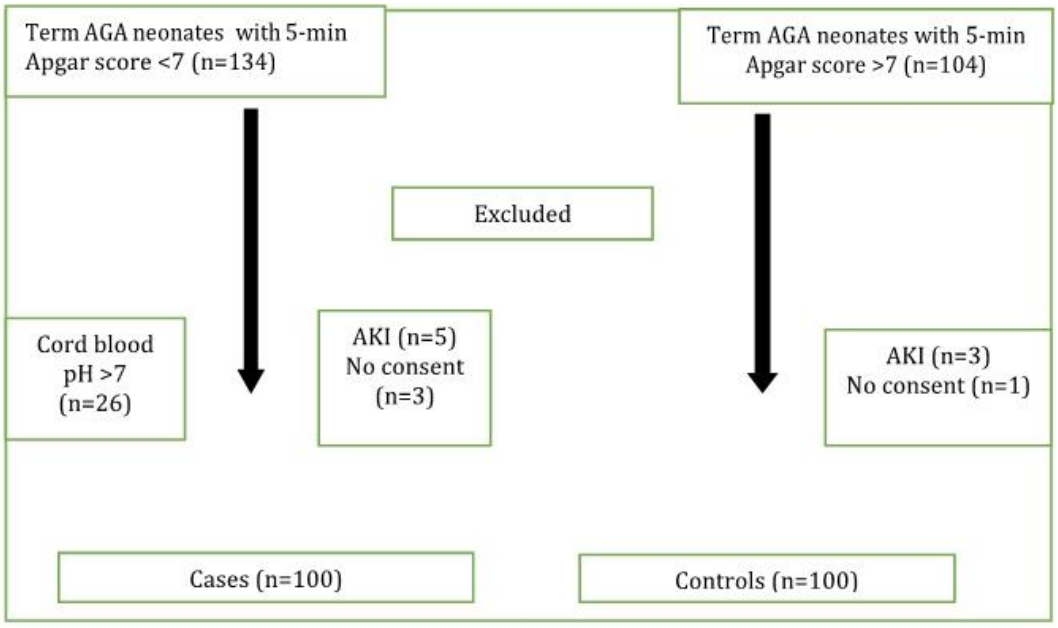

Figure 1. Study flow chart

serum glutamic pyruvate transaminase, serum glutamic-oxaloacetic transaminase, alkaline phosphatase (ALP), creatine phosphokinase myocardial band, and LDH were assessed upon admission. The cord blood was collected soon after resuscitation, and blood gas analysis was performed on the ABG machine (Roche). A total of 5 ml urine sample was collected from all enrolled neonates within 24 h of life using a sterile disposable pediatric urine collection bag (100 ml capacity) attached to the perineum (Romsons Company, India). The sample was then transferred to the laboratory for the analysis of the urinary UA/Cr ratio by a Vitros 250 Dry chemistry autoanalyzer using the enzymatic colorimetric assay, as well as Uricase (9) and Jaffe's picrate analysis method10, respectively. The samples were stored at 0-4° Celsius until analysis was carried out.

Statistical analysis

The unpaired student t-test and the Chi-square test were used to compare the mean values of the biochemical parameters and categorical variables, respectively. Receiver operating characteristic (ROC) curve analysis was also utilized to determine the cut-off value of urinary UA/Cr ratio using area under the curve (AUC), sensitivity, and specificity. Moreover, regression analysis with a scatter diagram was employed to find out the correlation of this ratio with Apgar score and cord blood pH. The data were compiled and analyzed using Microsoft Excel and Windows SPSS software (version 25).

Results

The demographic characteristics of the study population are summarized in Table 1. Cases and controls were statistically matched for GA and weight. Mean Apgar scores in cases and controls were 3.46±0.83 vs. 7.43±0.49 at 1 min, 5.12±0.78 vs. 8.76±0.42 at 5 min, and 7.21±0.80 vs. 9.00±0.0 at 10 min, respectively. Differences in the mean Apgar scores between cases and controls were statistically significant (P<0.0001). The mean cord blood pH was 6.82+0.03 in cases, while in controls, it was obtained at 7.37+0.04 (P<0.001). Furthermore, the mean urinary UA/Cr ratios were estimated at 3.41±0.68 in cases and 1.99±0.23 in controls (P<0.0001; Table 1).

In total, 49% of the cases were in stage II HIE, followed by 26% in stage III HIE, and 16% in stage I HIE. In 9% of the cases, there was no HIE, and none of the controls had HIE. Furthermore, 64% of the neonates had urinary UA/Cr ratio between 2.5 and 3.49, followed by 26% with a ratio between 3.5 and 4.49 in cases, whereas 94% of the controls had urinary UA/Cr ratio between 1.5 and 2.49 (P<0.05; Table 2).

Urinary UA/Cr ratio in the neonates with Stage III HIE was more than that of those in stages II and I HIE. It should be noted that this ratio was statistically significant (4.05 vs. 3.32 vs. 3.0) (P<0.0001). This ratio increases as the HIE staging of the asphyxiated newborn worsens (Table 3). Similar results were found when urinary UA/Cr ratio correlated with cord blood pH (Figure 2). The correlation of the urinary UA/Cr ratio with the 5-min Apgar score was statistically significant (r=-0.8806, P<0.001) (Figure 3). As the 5-min Apgar score decreases, the urinary UA/Cr ratio increases.

Table 1. Demographic characteristics of the study population

Parameter	Cases (n= 100)	Controls (n=100)	P-value
Male: Female ratio	1:1	0.92:1	0.887
Mean birth weight	2.87±0.27 kg	2.90±0.21 kg	0.459
Mean gestational age	37.83±1.21 weeks	37.51±0.64 weeks	0.25
Mean Apgar score			
1 min	3.46±0.83	7.43±0.49	<0.0001
5 min	5.12±0.78	8.76±0.42	
10 min	7.21±0.80	9.00±0.0	
Mean cord blood pH	6.82±0.03	7.37±0.04	< 0.001
Mean UA/Cr ratio	3.41±0.68	1.99±0.23	< 0.0001

Table 2. Urinary uric acid and creatinine ratio of the study population

Urinary uric acid and creatinine ratio	Cases (n=100)	Controls (n=100)	Total (n= 200)
< 1.5	00 (00%)	03 (03%)	03 (1.5%)
1.5-2.49	01 (01%)	94 (94%)	95 (47.5%)
2.5-3.49	64 (64%)	03 (03%)	67 (33.5%)
3.5-4.49	26 (26%)	00 (00%)	26 (13%)
≥4.5	09 (09%)	00 (00%)	09 (4.5%)
Total	100	100	200

According to the ROC curve analysis, the cut-off level of urinary UA/Cr ratio was >2.5, and AUC was determined at 0.996 with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 98%. Similarly, the cut-off value and cord blood pH at 5-min Apgar score were <7 and ≤ 7.0, respectively, which obtained sensitivity, specificity, PPV, and NPV of 100% with AUC of 1.00 (Table 4, Figure 4).

Table 3. Correlation of hypoxic-ischemic encephalopathy staging with urinary uric acid and creatinine ratio

HIE Stage	Cases (n=100)	Controls (n=100)	P-value
No	2.75±0.33	1.99±0.23	
Stage I HIE	3.00±0.24	-	
Stage II HIE	3.32±0.54	-	<0.0001
Stage III HIE	4.05±0.69	-	

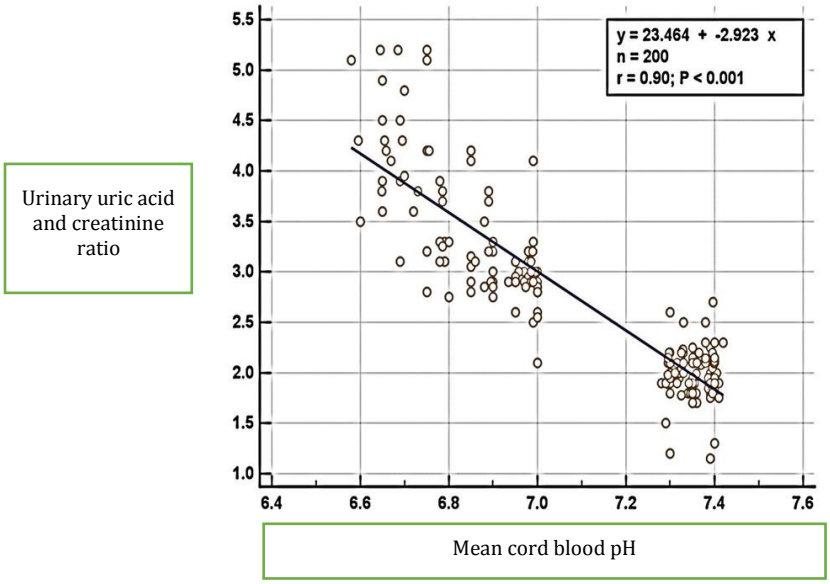

Figure 2. Correlation of urinary uric acid and creatinine ratio with mean cord blood pH

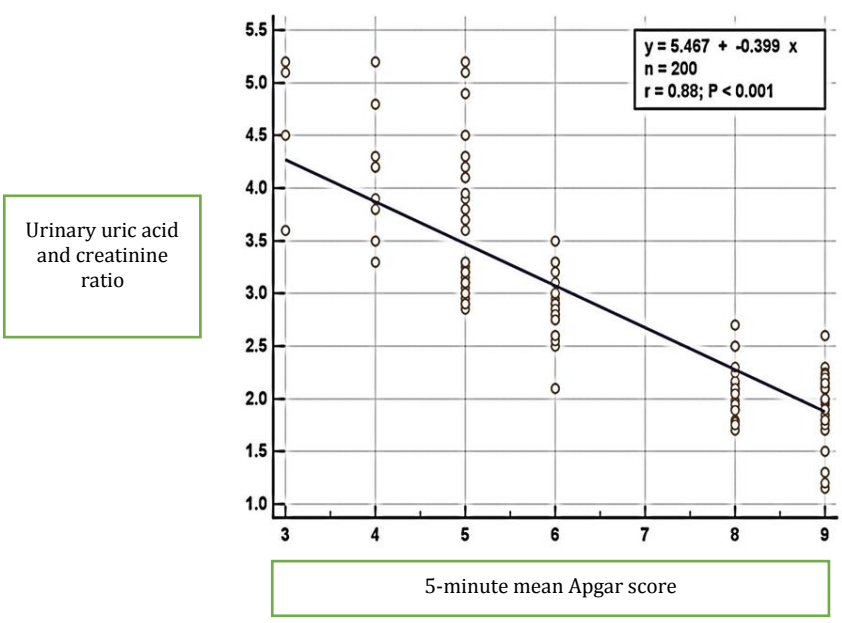

Figure 3. Correlation of urinary uric acid and creatinine ratio with 5-minute mean Apgar score

Table 4. Sensitivity, specificity, area under the curve of the cord blood pH, Apgar at 5-min, as well as urinary uric acid and creatinine ratio of the study population

Parameters	Cut off	Sensitivity	Specificity	AUC	PPV	NPV	P-value	95% CI of AUC
PH	≤7.0	100%	100%	1.000	100%	100%	<0.0001	0.98-1.00
Apgar at 5 min	<7	100%	100%	1.000	100%	100%	<0.0001	0.98-1.00
UA/Cr	>2.5	98%	98%	0.996	98%	98%	<0.0001	0.974-1.00

AUC: area under curve, PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval

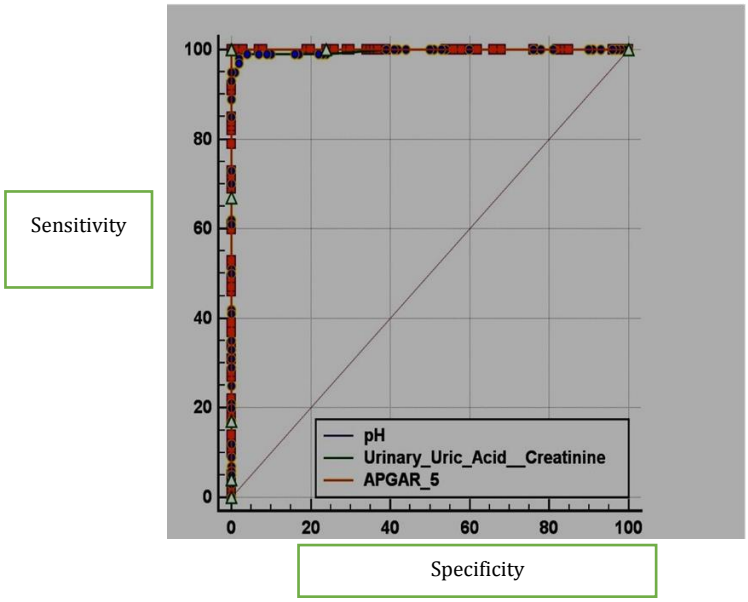

Figure 4. ROC curve depicting cord blood pH, 5-min Apgar, urinary uric acid, creatinine ratio as a marker of perinatal asphyxia

Discussion

The present study assessed the urinary UA/Cr ratio to distinguish asphyxiated from non-asphyxiated term neonates. This test is routinely available in most centers; moreover, it is cheap and non-invasive. Many studies (11-21) have demonstrated that urinary UA/Cr ratio can be utilized as a marker of PA which biochemically supports the diagnosis and identifies the severity of HIE. In our study, the mean urinary UA/Cr ratio in cases was more than that in controls; in addition, their correlation was statistically significant (P<0.001). Basu P et al. (11) and Bhongir AV et al. (12) also showed in their study that the urinary UA/Cr ratio was significantly more in cases than controls, which was consistent with the results of our study. In our study, the mean urinary UA/Cr ratio was statistically and significantly more in cases with stage III HIE, compared to those in stages II and I HIE, which shows that as the HIE staging of the asphyxiated newborn worsens, urinary UA/Cr ratio increases.

Similarly, Nariman S et al. (13) in their study on 362 preterm infants found that the mean urinary UA/Cr ratio in cases was significantly higher than that in normal neonates (3.30±1.95 vs. 1.36±0.42; P=0.0001). They concluded that the urinary UA/Cr ratio may be a noninvasive and inexpensive marker for detecting the outcome of ill asphyxiated neonates admitted in NICU. Varma V et al. (14) also reported that urinary UA/Cr ratio in an early void urine sample was a reliable method for diagnosing PA in the NICU within 24 h after birth. In a review article on 14 studies by Bellos et al. (15), it was found that urinary UA/Cr ratio was a simple and fast biomarker to determine neonatal asphyxia.

The cut-off value of the urinary UA/Cr ratio was determined at >2.5 with ROC curve analysis that each had a sensitivity, specificity, PPV, and NPV of 98%. Bhongir AV et al. (12) in their study found the cut-off value of urinary UA/Cr ratio of >2.43 which had 80% sensitivity and 87.5% specificity with the AUC of 0.84. Banupriya C et al. (16) found that the cut-off value of this ratio to label PA was >2.34. The cut-off values in their studies were also in concordance with those in our findings. A statistically significant correlation (r=-0.8951, P<0.001) was also observed between urinary UA/Cr ratio and cord blood pH (higher urinary UA/Cr ratio leads to lower cord blood pH). Neonates with a urinary UA/Cr ratio of > 4.5 had

the lowest mean cord blood pH (6.68+0.35). In a study by Bhongir AV et al. (12), urinary UA/Cr ratio showed a significant negative correlation with cord blood pH (r=-0.63, P=0.002), and the results of their study were in line with the findings of our study; however, the present study showed a better correlation between the two variables. Patel KP et al. (17) also found a negative correlation between arterial blood pH and urinary UA/Cr ratio; however, their correlation was not statistically significant (r=-0.18).

A statistically significant negative correlation was found between urinary UA/Cr ratio and 5-min Apgar score (r=-0.8806, P<0.001). Cases with a urinary UA/Cr ratio of >4.5 had a lower 5-min mean Apgar score of 3.89+0.75. In a study by Bader D et al. (18), urinary UA/Cr ratio significantly correlated with a 5-min Apgar score (r=- 0.86, P<0.05). Basu P et al. (11) showed a significant negative linear correlation between urinary UA/Cr ratio and Apgar score (r=-0.857, P<0.01), which was similar to that in the present study. A similar negative correlation between a 5-min Apgar score and urinary UA/Cr ratio was observed in the studies conducted by Patel KP et al. (17) and Sreekrishna YE et al. (19). A low Apgar score is commonly used as an indicator of asphyxia in infants; however, it may not be available in many cases (22). This study has shown a significant correlation of the urinary UA/Cr ratio with the severity of HIE so that this ratio can be used in extramural neonates to detect PA, where the Apgar score, as well as the cord blood pH, is not known. However, this is another area of research and requires more studies to apply this ratio on a routine basis in outborn babies.

Conclusion

The urinary UA/Cr ratio is a non-invasive, cheap, and reliable marker of PA with good predictive value as found in our study. It can also be used to identify PA in extramural babies when the Apgar score and cord blood pH are not available in most cases. However, the use of this ratio on a routine basis and outborn babies requires more studies with large samples.

Acknowledgments

The study protocol was approved by the Institutional Ethics Review Committee of Dr. S N Medical College, Jodhpur, India. The authors thank all parents who gave consent for the study.

Conflicts of interest

None.

Funding

None.

Authors' contributions

Dr. Kartika Gulati was responsible for data collection, interpretation of the results, review of the literature, and manuscript preparation. Dr. Shree Krishan Vishnoi was responsible for the concept, coordination, review of the literature, and manuscript preparation. Dr. Sandeep Choudhary was responsible for data analysis, interpretation of the results, as well as manuscript preparation and revision. Dr. Rakesh Jora was responsible for the concept and design of the study.

References

1. World Health Statistics. 2013.
2. Porter KB, O'brien WF, Benoit R. Comparison of cord purine metabolites to maternal and neonatal variables of hypoxia. Obstet Gynecol. 1992; 79(3):394-7.
3. Palmer C, Vannucci RC, Towfighi J. Reduction of perinatal hypoxic-ischaemic brain damage with allopurinol. Pediatr Res. 1990; 27(4):332-6.
4. Poulsen JP, Rognum TO, Sagustad OD. Changes in oxypurine concentrations in vitreous humor of pigs during hypoxaemia and post-mortem. Pediatr Res. 1990; 28(5):482-4.
5. Hankins GD, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. Obstet Gynecol. 2003; 102(3):628-36.
6. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL, et al. Modified RIFLE criteria in critically ill children with acute kidney injury. *Kidney Int*. 2007; 71(10):1028-35.
7. Bansal SC, Nimbalkar SM. Updated neonatal resuscitation guidelines 2015–major changes. Indian Pediatr. 2016; 53(5):403-8.
8. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol. 1976; 33(10):696-705.
9. Kalckar HM. Differential spectrophotometry of purine compounds by means of specific enzymes. 1. Determination of hydroxypurine compounds. *J* Biol Chem. 1947; 167(2):429-43.
10. Knapp ML, Mayne PD. Development of an automated kinetic Jaffe method designed to minimise bilirubin interference in plasma creatinine assays. Clin Chim Acta. 1987; 168(2):239-46.
11. Basu P, Som S, Choudhuri N, Das H. Correlation between Apgar score and urinary uric acid to creatinine ratio in perinatal asphyxia. Indian J Clin Biochem. 2008; 23(4):361-4.
12. Bhongir AV, Yakama AV, Saha S, Radia SB, Pabbati J. The urinary uric acid/creatinine ratio is an adjuvant marker for perinatal asphyxia. Eur J Pharm

Med Res. 2015; 2(5):520-528.
13. Nariman S, Mosayebi Z, Sagheb S, Rastad H, Hosseininodeh SS. Urinary uric acid/creatinine ratio as a marker of mortality and unfavorable outcome in NICU-admitted neonates. Iran J Pediatr. 2016; 26(4): e5739.
14. Vandana V, Amit V, Meena V, Anuradha B, Vivek B, Deepak V, et al. Study of basic biochemical and haematological parameters in perinatal asphyxia and its correlation with hypoxic ischemic encephalopathy (HIE) staging. J Adv Res Biol Sci. 2011; 3(2):79–85.
15. Bellos I, Fitrou G, Pergialiotis V, Perrea DN, Papantoniou N, Daskalakis GJTJoM-F. Random urine uric acid to creatinine and prediction of perinatal asphyxia: a meta-analysis. J Matern Fetal Neonatal Med. 2019; 32(22):3864–70.
16. Banupriya C, Doureradjou P, Mondal N, Vishnu B, Koner BC. Can urinary excretion rate of malondialdehyde, uric acid and protein predict the severity and impending death in perinatal asphyxia. Clinical Biochemistry 2008; 41(12):968-73.
17. Patel KP, MaKadia MG, Patel VI, Nilayangode HN, Nimbalkar SM. Urinary uric acid/creatinine ratio-a marker for perinatal asphyxia. J Clin Diagn Res. 2017; 11(1):SC08-10.
18. Bader D, Gozal D, Weinger-Abend M, Berger A, Lanir A. Neonatal urinary uric acid/creatinine ratio as an additional marker of perinatal asphyxia. Eur J Pediatr. 1995; 154(9):747-9.
19. Sreekrishna Y, Eregowda *A,* Sharma HL. Study of urinary uric acid to creatinine ratio as a biochemical marker of perinatal asphyxia and its correlation with Apgar Score. Int J Contemp Pediatrics. 2018; 5(4):1485.
20. Choudhary L, Palsania S, Berwal P, Sauparna C, Maheshwari A. Study of urinary uric acid and creatinine ratio as a marker of perinatal asphyxia and its correlation with diferent stages of hypoxic ischemic encephalopathy. J Preg Child Health. 2017; 4:1-4.
21. Krishnana EPV, Sekar SP, Ponnusamy V. Study of urinary uric acid and creatinine ratio as a marker of neonatal asphyxia for babies born in a tertiary care hospital. Int J Res Med Sci. 2017; 5(12): 5418–23.
22. American Academy of Pediatrics; Committee on Fetus and Newborn; American College of Obstetricians and Gynecologists; Committee on Obstetric Practice. The APGAR score. Adv Neonatal Care. 2006; 6(4):220-23.