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THE URINARY URIC ACID/CREATININE RATIO IS AN ADJUVANT MARKER FOR PERINATAL ASPHYXIA.

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ABSTRACT

Objective: To assess the urinary uric acid/creatinine ratio (UA/Cr) in relation to Apgar score and cord blood gas analysis in identification of perinatal asphyxia and to define the cutoff values. ***Design*:** case control study. ***Setting*:** The newborns admitted in the department of pediatrics and NICU of Mediciti Institute of Medical Science, Ghanpur, Medchal mandal, Telangana from May-July 2011 were enrolled. ***Participants/patients*:** The study was conducted on 31 (18 males, 13 females) controls and 18 (12males, 6 females) asphyxiated neonates. ***Outcome Measure(s)*:** 5ml of arterial cord blood of newborn collected at the time of birth and spot urine samples were collected within 24-72 hours of life. Cord blood gas analysis were done immediately and Urinary uric acid was measured by modified Uricase method, urinary creatinine by modified kinetic Jaffe's reaction. ***Results:*** The mean urinary uric acid and creatinine ratio (2.58± 0.48 vs 1.89 ± 0.59) is significantly higher in Asphyxiated group than in the control group. The umbilical cord blood pH had significant positive correlation with 1st minute Apgar score (r= 0.41, p=0.003), 5th minute Apgar (r= 0.44, p=0.002), while urinary UA/Cr ratio had significant negative correlation with cord blood pH (r= -0.63, p=0.002). Urinary UA/Cr ratio with criterion of >2.43 had 80% sensitivity, 87.5% specificity with AUC of 0.84 (p=0.003) had a better predictive value. ***Conclusions*:** Urinary UA/Cr ratio is easy, non-invasive, painless and

economical adjuvant parameter with better predictive value for diagnosing perinatal asphyxia with simple diagnostic equipment.

KEY WORDS: apgar score, asphyxia, cord blood pH, urinary uric acid and creatinine ratio.

INTRODUCTION

Perinatal asphyxia is a condition where there is an impaired gas exchange leading to hypoxemia, hypercapnea, and acidosis in fetus or neonate. The incidence of perinatal asphyxia is 1-1.5% live birth. Statistics in India suggests it to be the main cause of mortality (28.8%), morbidity and chief cause of stillbirth (45.1%).[1]

The most commonly used diagnostic and prognostic indicator to assess asphyxia in neonate is apgar score. The 1-minute Apgar score reflects the need for immediate resuscitation. The change in score between 1 and 5 minutes is an useful index for the effectiveness of resuscitative efforts. But Apgar score alone does not predict neurologic outcome like cerebral palsy and as it is influenced by various factors like immaturity, fetal malformations, maternal medications and infection. Whereas Apgar score and umbilical artery blood pH both predict the neonatal mortality in term and preterm infants.[2] Indicators such as pH, lactates and base deficits subside with the establishment of respiration, and moreover these techniques are costly and sophisticated.

Accurate assessment of late neurological sequelae has failed by implementing strategies such as fetal heart monitoring[3], apgar score[2], while analysis of xanthine, hypoxanthine, neuron-specific enolase , brain-specific creatine kinase and inflammatory cytokines (tumor necrosis factor-alpha, interleukin-1-beta, interleukin-8, interleukin-6) are time consuming, costly and not routinely available for clinical care.[4][5][6][7]

The high purine metabolism in asphyxiated neonates results in elevated levels of Xanthine, Hypoxanthine, Uric Acid and in presence of xanthine oxidase, the production of superoxide radicals and free radicals increases with Uric acid formation and hence can be used as a marker of tissue hypoxia.[8] Increased excretion of uric acid caused by metabolic changes, reflecting the cellular hypoxia has been reported by number of studies.[9][10] Some studies have reported higher urinary UA/Cr ratio in preterm and term infants with perinatal asphyxia than in normal infants.[11][12] Some studies have compared urinary UA/Cr ratio with apgar[13] and some with Cord blood pH[14] but none of them compared with both apgar score and cord

blood pH. Hence, this study was conducted to assess the urinary uric acid/creatinine ratio (UA/Cr) in relation to Apgar score and cord blood gas analysis, in identification of perinatal asphyxia and to define the cutoff values.

METHODS

After the approval of Institutional ethical committee, the case control study was conducted in the Pediatric and Biochemistry department of Mediciti Institute of Medical Science from May-July 2011. The newborns admitted in the department of pediatrics and NICU were enrolled for the present study. Written informed consent has been obtained from parents. The sample size was calculated to be 8 in each group based on Pallav basu et al study[13] (means of urinary UA/Cr ratio 3.1 and 0.9 was used with 90% power and alpha of 0.05).

The present study included 18 asphyxiated newborns as case group and 31 healthy newborns as a control group. The newborns were included in perinatal asphyxia group if the infant had an apgar score of <7 at 1st min or 5th min of birth, or cord blood pH < 7.00.[1][15] Newborns with congenital malformations, those suffering from anuria were excluded. Infants born to mother with history of febrile illness within 2 weeks before delivery and those receiving general anesthesia, diuretics, pethidine, and other drugs likely to cause depression in babies were excluded. Full-term infants born after an uncomplicated pregnancy with an apgar score of >7 at 5th min and with no signs of asphyxia were included in the control group.[16] A predesigned pretested proforma was used to record gestational age, birth weight and relevant perinatal history and clinical findings.

5ml of arterial cord blood has been collected after delivery following NCCLS (National Clinical Chemistry Laboratory Standards) guidelines.[17] The spot urine samples were collected within 24-72 hours of life in infants using sterile disposable pediatric urine collection bag (100ml capacity) attached to the perineum (Romsons Company-India). Since only a standard urine bag was available, it was used for all newborn infants, irrespective of birthweight. We encountered a problem with especially female infants during the urine collection due to the size of the bag; the sample was contaminated with meconium/fecal matter and hence discarded. This was because of the non-availability of urine bags as per birth weight and size of the babies similar to the availability of diapers. We could only collect 16 and 5 urine samples of Control and Asphyxiated group respectively.

Urinary uric acid was measured by modified Uricase method[18] and urinary creatinine by modified kinetic Jaffe's reaction on Dade Behring-dimension expand plus auto analyzer[19]. Cord arterial blood gas analysis was done on radiometer blood gas analyzer. All biochemical parameters were analyzed immediately. Measures have been taken for checking the kit to kit variability and the repeatability has been checked by duplicate testing. The intra and inter assay coefficients of variation for uric acid and creatinine were 2.2% and 3.5% respectively. The markers of heart rate, respiratory efforts, tone, reflex activity and color were used to establish apgar score at 1 minute and 5th minute of age (maximum score of 10) Asphyxia <7, moderate asphyxia 4-6 and severe asphyxia 0-3.[16]

Statistical analysis

The data was entered in MS-Excel database. After cleaning and validation, the data was analyzed using the statistical program MedCalc for PC, version 12.7.0 (MedCalc software, Belgium). Descriptive analyses for intergroup comparison were done using unpaired t test and chi square test. Pearson correlation coefficient was used to test the strength of association between cord blood pH and other variables. Receiver operating characteristic (ROC) plots were used to determine the cut-off values of various parameters. A p value of <0.05 was considered significant.

RESULTS

The study was conducted on 31 (18 males, 13 females) controls and 18 (12males, 6 females) asphyxiated neonates. The mean gestational age and birth weight of asphyxiated group (36.4 ± 3.33 weeks, 2.42 ± 0.52 kg) were significantly lower than the control group (37.9 ± 1.77 weeks, 2.79 ± 0.46 kg) respectively. (Table 1)

TABLE-1: Demographic characteristics of the study groups.

PARAMETER	ASPHYXIATED GROUP	CONTROL GROUP
Number(n)	18	31
No. of males	12	18
No. of females	6	13
Mode of delivery:		
A. Vaginal	15	24
B. C-section	3	7
Size for gestational age		
A. Small	14	15
B. Appropriate	4	16
C. Large	0	0

Gestational age (weeks) (mean ± SD)	36.4 ± 3.33	37.9 ± 1.77 (p=0.04)
Birth weight (kg) (mean ± SD)	2.42 ± 0.52	2.79 ± 0.46 (p=0.01)

Mean fetal heart rate did not differ between the two groups. While mean apgar score at 1st minute (6.3 ± 1.3 vs 7.6 ± 0.5), 5th minute (8.3 ± 1.1 vs 9.5 ± 0.7), cord blood pH (7.12 ± 0.09 vs 7.32 ± 0.08), pO2 (19.9 ± 7.2 vs 41.1 ± 16.4 mm Hg) were significantly lower in the asphyxiated group than in the control group respectively. The mean pCO2 (66.8 ± 8.1 vs 43.8 ± 9.2 mm Hg) and mean urinary uric acid and creatinine ratio (2.58± 0.48 vs 1.89 ± 0.59) were significantly higher in asphyxiated group than in the control group respectively. (Table 2)

Table 2: Comparison of various parameters between the control and asphyxiated group.

PARAMETER (mean ± sd)	AXPHYXIATED GROUP (N=18)	CONTROL GROUP (N=31)	P VALUE
FETAL HEART RATE (beats/min)	139.8 ± 6.6	140.5 ± 5.5	P = 0.71
APGAR 1ST MIN	6.3 ± 1.3	7.6 ± 0.5	P < 0.0001
APGAR 5TH MIN	8.3 ± 1.1	9.5 ± 0.7	P = 0.0001
CORD BLOOD PH	7.12 ± 0.09	7.32 ± 0.08	P < 0.0001
PO2 (mm Hg)	19.9 ± 7.2	41.1 ± 16.4	P < 0.0001
PCO2 (mm Hg)	66.8 ± 8.1	43.8 ± 9.2	P < 0.0001
URINARY URIC ACID/ CREATININE RATIO	2.58± 0.48 (n=5)	1.89 ± 0.59 (n=16)	P = 0.0280

The umbilical cord blood pH showed significant positive correlation with apgar score at 1st minute (r=0.41, p=0.0031), apgar 5th minute (r=0.44, p=0.0017). While urinary UA/Cr ratio showed significant negative correlation with cord blood pH. (r= -0.63, p=0.0022) (Table 3)

Table 3: Correlation of pH and Apgar Score At 1st min, 5th min, UA/Cr ratio.

PARAMETER	APGAR 1ST MIN	APGAR 5TH MIN	UA/Cr RATIO
pH	r=0.41	0.44	-0.63
p value	0.0031	P=0.0017	P=0.0022

Receiver operating characteristic (ROC) plots showed the cord blood pH with cut off value of ≤7.2 had highest predictive value for perinatal asphyxia (sensitivity 94.4%, specificity 96.8%, AUC 0.97 and p<0.0001). Urinary UA/Cr ratio with criterion of >2.43 had 80% sensitivity, 87.5% specificity with AUC of 0.84 (p=0.003) had a better predictive value than Apgar score 1st min and 5th min.(figure1,2) The Apgar score 1st min (cut off of ≤6) had a

better predictive value with higher AUC of 0.81, specificity of 100% than Apgar at 5th min (cut off ≤8), specificity of 87.1%, AUC of 0.79 (p<0.0001) and but had similar sensitivity of 55.6%. (Table 4)

Table 4: The predictive values of biochemical parameters for perinatal asphyxia- cord blood pH, APGAR 1st min & 5th min, and urinary UA/Cr ratio.

PARAMETER	CUT OFF VALUE	SENSITIVITY	SPECIFICITY	AUC	P VALUE	95% CONFIDENCE INTERVAL
pH	≤7.2	94.4	96.8	0.97	<0.0001	0.87 to 0.99
APGAR 1st min	≤6	55.6	100	0.81	<0.0001	0.67 to 0.91
APGAR 5th min	≤8	55.6	87.1	0.79	<0.0001	0.65 to 0.89
UA/Cr ratio	>2.43	80	87.5	0.838	0.003	0.61 to 0.96

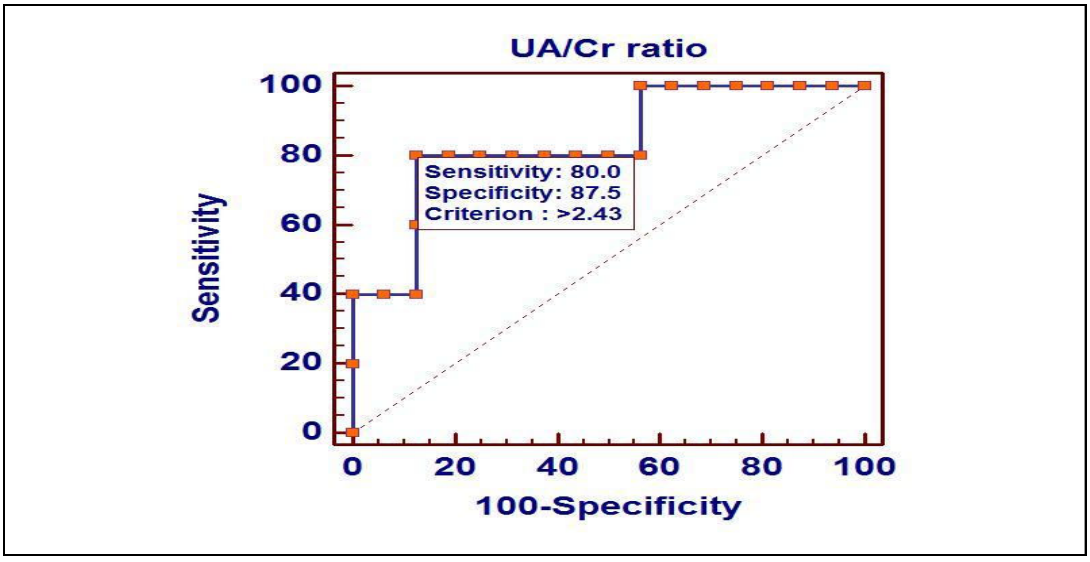

Figure 1: ROC curve of urinary UA/Cr ratio for perinatal asphyxia.

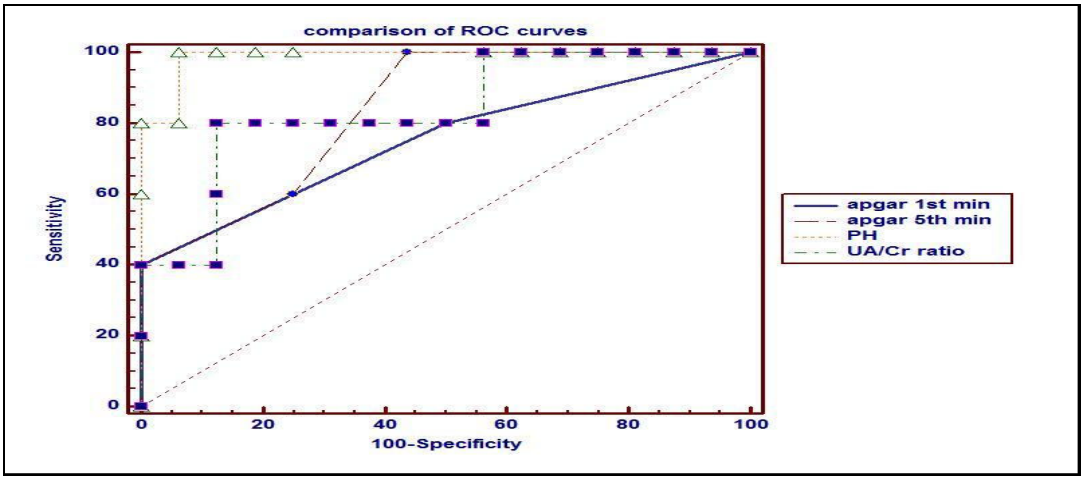

Figure 2: Comparison of ROC curves- Apgar 1st min, 5th min, cord blood pH and urinary UA/Cr ratio.

DISCUSSION

Perinatal asphyxia is a dreadful condition because of its potential for causing permanent damage, even death of the fetus or new born infant. Increased urinary UA/Cr ratio (as a sign of increase ATP degradation) can be used as a valuable indicator of the severity of tissue hypoxia in perinatal asphyxia.[20]

In this study urinary UA/Cr ratio was significantly higher in asphyxiated group than in the control group. Similar findings are seen in various studies that have reported the urinary UA/Cr ratio was significantly higher in both term and preterm infants with perinatal asphyxia than healthy full term infants.[21][13][14] Akisu et al and vandana Varma et al also reported that urinary UA/Cr ratio in an early void urine sample is a reliable method for diagnosing perinatal asphyxia in a neonatal Intensive Care unit within 24 hours after birth and correlates with the severity hypoxic ischemic encephalopathy (HIE).[12][22] Present study showed that the predictive value of perinatal asphyxia was significantly higher for cord blood pH followed by urinary UA/Cr ratio than Apgar scores of 1 minute and 5th minute. And the urinary UA/Cr ratio was significantly negatively linearly correlated with umbilical cord blood pH. Pallav basu et al reported significant negative linear correlation between urinary UA/Cr ratio and the Apgar score ($r = -0.857$, $p < 0.001$).[13] While in the present study there is negative linear correlation between Apgar score 1st minute, and urinary UA/Cr ratio ($r= -0.33$, $p=0.14$) and between Apgar score 5th minute and urinary UA/Cr ratio ($r= -0.26$, $p=0.25$) but not statistically significant.

Our study emphasizes that urinary UA/Cr ratio in spot urine sample within 24-72 hours of life is a better marker for Perinatal Asphyxia, with cutoff value of >2.43 had 80% sensitivity, 87.5% specificity with AUC of 0.84 ($p=0.003$). Urinary UA/Cr ratio is easy, non-invasive, painless and economical adjuvant parameter for diagnosing perinatal asphyxia with simple diagnostic equipment. Combined use of cord blood pH, Apgar scores and UA/Cr ratio can help in early decision making about the level of care the new born requires.

There is a need for larger population based prospective study to determine cutoff values of urinary UA/Cr ratio for the severity of perinatal asphyxia.

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