Original Article

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

Biochemistry Section

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ABSTRACT

Introduction: Perinatal asphyxia is a very common problem which significantly contributes to neonatal morbidity and mortality, contributing to almost 20% of neonatal deaths in India. It is an important cause of static developmental and neurological morbidity. Early identification of asphyxiated babies to take timely appropriate measures is our priority. Several measures like foetal heart monitoring, Appearance, Pulse, Grimace, Activity, Respiration score (APGAR), Cord blood pH, Computerised Tomography (CT), Magnetic Resonance Imaging (MRI), Doppler studies etc., are already in use for early identification of asphyxia; but they are costly and have several other limitations. Urinary Uric Acid and Creatinine Ratio (UA/Cr) is a non-invasive test, easy to conduct in resource poor setup and an early marker of asphyxia, which prompted the authors to conduct the present study.

Aim: To assess the correlation of urinary uric acid and creatinine ratio with perinatal asphyxia and observe any correlation between this ratio with the severity of Hypoxic Ischaemic Encephalopathy (HIE).

Materials and Methods: This was a prospective case control study conducted in Patna Medical College and Hospital, Patna, Bihar, India, between January 2019 to March 2020. A total of 100 asphyxiated term neonates as cases and 100 non-asphyxiated term neonates as controls were included in the study. Spot urine samples were collected and urinary uric acid and creatinine were estimated. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS version. 16.0) software and p-value <0.05 was considered as statistically significant.

Results: A higher urinary UA/Cr ratio of 2.99±0.14 was seen in case group compared to control group (0.77±0.03) and the higher ratio was also associated with significant neurological abnormalities. A highly significant difference was noted between case and control i.e., p<0.001 with 95% CI of 1.95-2.49.

Conclusion: Urinary uric acid and creatinine ratio enables early recognition of asphyxial injury and also is a good indicator for subsequent morbidity and mortality. These measurements require simple instruments with low cost involvement. The correlation between the urinary UA/Cr ratio and the severity of HIE provides an indication for the degree of injury at an early stage when other quantitative methods cannot be carried out frequently. Therefore, this ratio can be used as an early marker of asphyxial injury and can predict neurological deficit.

Keywords: Cord blood pH, Hypercapnea, Tissue hypoxia

INTRODUCTION

Perinatal asphyxia is a condition in which there is an impaired gas exchange leading to hypoxaemia, hypercapnia, and acidosis in fetus or neonate. Globally, it accounts for about 23% of all neonatal deaths [1]. As per data from National Neonatal Perinatal database (NNPD), about 28.8% of neonatal deaths in India is due to perinatal asphyxia, about 84 out of 1000 newborn babies had one minute APGAR score less than seven and 14 out of 1000 among them suffered HIE [2]. Perinatal asphyxia usually has adverse effects on all major organs, resulting in renal, neurologic, cardiac and pulmonary dysfunction in 50%, 28%, 25% and 23%, respectively, in term neonates [3,4].

Early identification of asphyxiated babies to take timely appropriate measures to reduce neonatal morbidities is our priority. Several measures like foetal heart monitoring, APGAR score, cord blood pH, CT, MRI, Doppler studies etc. are in use for early identification of perinatal asphyxia; but they all have several limitations necessitating a better and easily available measure.

It has been observed in various studies that in the condition of uninterrupted tissue hypoxia and reperfusion injury, hypoxanthine is oxidized to xanthine and uric acid in the presence of the enzyme xanthine oxidase resulting in an increase in uric acid production and excretion in urine [5-7]. In one study Basu P et al., has observed that in asphyxiated neonates, urinary UA/Cr ratio within 24 hours of birth was higher than non-asphyxiated neonates [8]. This study was further supported by studies done out by Akishu M et al., Bader D et al., and Bhanupriya C et al., [9-11]

Estimation of urinary uric acid and creatinine ratio is a simple, non-invasive, painless and easily available test. Keeping this in mind, the present study was designed to know the effect of perinatal asphyxia on urinary UA/Cr ratio and to correlate the level of urinary UA/Cr ratio with HIE grading.

MATERIALS AND METHODS

This was a prospective case control study conducted on full term asphyxiated and non-asphyxiated neonates admitted to Neonatal intensive care unit and post-natal ward of Patna Medical College and Hospital, Patna, Bihar, India, between January 2019 to March 2020. A sample size of 50 was sufficient for this study without type II error to estimate urinary UA/Cr ratio, but we took a sample size of 100 to increase the accuracy of the results. A total of 100 asphyxiated term neonates as cases (study group) and 100 non-asphyxiated term neonates as controls (control group) were included in the study.

The babies were examined on day one of admission. Written informed consent was taken from the parents. Research work was started after applying for Ethical clearance from Institutional Ethical committee of Patna Medical College, with its reference no. MF/682.

Inclusion Criteria

For study group:

1. Gestational age more than 37 completed weeks.
2. Birth weight ≥2.5 kg.
3. APGAR score less than 7 at one minute after birth [12].
4. Delayed cry more than five minutes.
5. Need for positive pressure ventilation for more than one minute.
6. Mild, moderate or severe HIE as defined by Sarnat HB and Flores-Sarnat L [13].
7. First day of life.

For control group:

1. Gestational age more than 37 completed weeks.
2. Birth weight ≥2.5 kg.
3. No signs of birth asphyxia.
4. Baby cried immediately after birth.
5. APGAR score more than 7 at one minute.
6. Mothers with no history of any medications which could affect the neonatal health.
7. More than 24 hours of birth.

Exclusion Criteria

1. Any congenital malformations.
2. Newborns of mothers having preeclampsia/eclampsia.
3. Haemolytic disease of newborn.
4. Mother taking any medications which could affect the neonatal health.
5. More than 24 hours of birth.

Detailed antepartum and intrapartum history of mothers were taken. Birth events, APGAR score, sex and weight of the babies were recorded. Gestational age was assessed by new Ballard scoring system [14]. Thorough clinical and neurological examination was done for all the neonates included in both the groups.

Urine samples collection: Urine samples were collected using sterile urine collection bags within 24 hours of life and analysed immediately in the laboratory.

Analysis of urinary samples: Urinary uric acid was estimated by autoanalyser by enzymatic assay- Uricase method [15]. Urinary creatinine was estimated by the same instrument by using modified kinetic Jaffe's method [16].

STATISTICAL ANALYSIS

The data obtained were statistically analysed using Microsoft Excel and SPSS software version 22 (using SPSS version 16.0; SPSS. Chicago, IL, USA). Descriptive statistics viz., number, percentage, range, mean±SD were utilised to describe the findings. Means were compared using Pearson correlation coefficient and Independent sample t-test, where applicable. Additionally, Standard Error (SE) and 95% Confidence Interval (CI) were also calculated for mean and p-value. A p-value <0.05 was considered as statistically significant.

RESULTS

A total of 100 asphyxiated newborns were examined who qualified the inclusion criteria. Their urinary samples were collected and uric acid and creatinine were estimated. Hundred newborns who qualified for inclusion in control group were similarly examined and their urinary samples were collected and uric acid and creatinine were estimated. There were 58 male babies and 42 female babies in study group, while 62 male and 38 female babies in control group. Birth weights in study and control groups were almost similar. It was in the range of 2.5 to 3.0 kg in 78% and 76% of study and control groups, respectively. It ranged between 3.1 and 3.5 kg in 20% and 22% of study and control groups, respectively. Only 2% newborns weighed more than 3.5 kg in both groups. No statistical significance could be attached to these baseline information. Details of baseline characteristics are given in [Table/Fig-1,2].

	Study group		Control group	
Sex	Male	Female	Male	Female
(n)	58	42	62	38

[Table/Fig-1]: Sex of babies in study and control group.

A total of 68% babies of study group and 78% of control group were born to primiparous mothers, while 32% and 22% to multiparous mothers, respectively. Lower Segment Caesarean Section (LSCS) delivery was 16% more in control group mothers. Timely intervention and opting for LSCS seems to play a definite role in minimising the neonatal asphyxia and its consequences. Comparison of various birth related parameters between the control and asphyxiated group are given in [Table/Fig-3,4].

Weight (Kg)	Study group (%)	Control group (%)
2.5-3	78	76
3.1-3.5	20	22
>3.5	02	02

[Table/Fig-2]: Birth weight of babies in study and control group.

Parity	Study group (%)	Control group (%)
Primipara	68	78
Multipara	32	22

[Table/Fig-3]: Parity of mothers of babies in study and control group.

Mode of delivery	Study group (%)	Control group (%)
Vaginal	84	68
LSCS	16	32

[Table/Fig-4]: Mode of delivery of babies in both study and control group. LSCS (LowerSsegment Caesarean Section) includes both elective and emergency).

A negative correlation was observed for UA/Cr ratio between two groups (case and control) and the r-value (-0.163) was not significant (p=0.258) at 95% CI. However, highly significant difference was noted between case and control i.e., p<0.001 with 95% CI of 1.95-2.49 [Table/Fig-5].

Sample	Range (Max-Min)	Mean (SE)	Correlation	Significance (95% CI)
Case	4.16 (4.94-0.78)	2.99 (0.14)	-0.163 (P=0.26)	p<0.001 (1.95-2.49)
Control	0.72 (1.14-0.42)	0.77 (0.03)		

[Table/Fig-5]: Observation of urine uric acid/creatinine ratio in study group and control group.

A highly significant difference was observed between HIE Grade I and Grade II <0.000 (-1.37 to -0.67) for urinary uric acid and creatinine ratio in study group. Similar observation was made between Grade I and Grade III p<0.001 (-2.31 to -1.63) and Grade II and Grade III p<0.001 (-0.93 to -0.49), respectively, in study group [Table/Fig-6,7].

HIE grading	Percentage	Mean (SE)
Non HIE	30	1.99 (0.16)
Grade I	22	2.53 (0.76)
Grade II	32	3.36 (0.10)
Grade III	16	4.39 (0.13)

[Table/Fig-6]: Distribution of HIE grading in study group.
*SE: Standard error

Grading		I HIE	II HIE	III HIE
I	HIE	NA	p<0.001 (-1.37 to -0.67)	p<0.001 (-2.31 to -1.63)
II	HIE	p<0.001 (-1.37 to -0.67)	NA	p<0.001 (-0.93 to -0.49)
III	HIE	p<0.001 (-2.31 to -1.63)	p<0.001 (-0.93 to -0.49)	NA

[Table/Fig-7]: Pearson correlation of urinary uric acid and creatinine ratio with HIE grading of neonates in study group.
Significant- p (95% CI)

DISCUSSION

Perinatal asphyxia is a common neonatal problem contributing significantly to neonatal mortality and morbidity. To diagnose perinatal asphyxia various modalities like cranial ultrasonography, tomography, MRI, somatosensory evoked potentials etc., are available. But these modalities are not of much diagnostic importance in first 24 hours of life. In various previous works urinary uric acid and creatinine ratio appeared as an early marker of hypoxic ischaemic brain injury and a

significantly elevated level had been observed in asphyxiated neonates [17,18]. Further in the neonates diagnosed with HIE, a significant association was observed between urinary UA/Cr ratio and severity of encephalopathy. Characteristics like sex, parity, mode of delivery, birth weight of neonates are similar in this study as in various other studies [19,20]. No significance could be attributed to these findings because of similar profile in both study and control population. A higher incidence of LSCS in study population with no asphyxia can be inferred as timely interference in birth processes could minimise the incidence of birth asphyxia and its consequences, although statistically we could not find significant data [Table/Fig-1-4].

In this study, urinary UA/Cr ratio was significantly higher in study group than the control group with a high significance [Table/Fig-5]. Overall, these findings were in accordance with findings reported by Shreekrishna Y et al., and Choudhary L et al., [17,18]. Shreekrishna Y et al., had observed urinary UA/Cr ratios significantly high in cases (asphyxiated) than controls (normal newborns), (mean urinary UA/Cr ratio in cases was 2.8±0.9 and in controls was 0.8±0.2; p<0.001) [17], while Choudhary L et al., had found the mean urinary uric acid and creatinine ratio of 2.68±1.06 in study group and 0.79±0.36 in control group (p-value=0.0001) [18]. Basu P et al., in a similar study found significantly higher urinary UA/Cr ratio in cases than controls (3.1±1.3 vs. 0.96±0.54; p<0.001) [8]. Our findings were also consistent with the previously reported studies by Bader D et al., and Vandana V et al., [10,19].

In the present study, a highly significant difference was observed between HIE Grade I and Grade II for UA/Cr in the study group. Similar correlation was observed between Grade I and Grade III, Grade II and Grade III, respectively [Table/Fig-6].

These basic findings are consistent to Banupriya C et al., [11]. They observed the Spearman's correlation depicting that urine uric acid creatinine ratio showed a significant positive correlation with HIE staging and significant negative correlation with APGAR score. Chen HJ et al., in their study had the observation correlating to our study [20]. Similar findings were reported by Suman KV et al., [21]. In their study, they observed that the mean values of urinary uric acid creatinine ratio in different stages of HIE showed increasing ratio with increasing stages of HIE with significantly higher ratios (P in stage II and III HIE (2.01±0.42 & 4.24±0.79) compared to control group (0.84±0.56) and also compared to stage I HIE (1.23±0.52) (p<0.0001). Choudhary L et al., had the similar findings where they observed the urinary UA/Cr ratios 3.61±0.61 in severe HIE, 2.95±0.98 in moderate HIE and those with mild HIE 2.64±0.25. They also observed the values of the UA/Cr ratios in the mild and moderate HIE groups were statistically significant (p<0.01) [18].

Limitation(s)

In this study, long term follow-up was not done, which might have indicated the benefit of this study on future growth and development of those asphyxiated babies and any significant shift in neurological status.

CONCLUSION(S)

A variety of indicators exist for birth asphyxia, currently. However, all of these have certain limiations to be used as predictors for subsequent morbidity. Urinary UA/Cr ratio is simple, economical, non-invasive and painless procedure, well-accepted to parents. It enables early recognition of asphyxial injury and also a good indicator for subsequent morbidity and mortality. Urinary uric acid and creatinine estimations need easily available reagents, simple instruments and techniques that can be acquired at low costs. The ratio is a good, simple screening test for the early assessment of perinatal asphyxia and can be used as a cost-effective, simple, quick and non-invasive parameter for early diagnosis of perinatal asphyxia.

Further the correlation between the urinary UA/Cr ratio and the severity of HIE provides an early indicator for the degree of injury at an early stage when other quantitative methods cannot be carried out frequently.

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