URINARY URIC ACID/CREATININE RATIO, B2-MICROGLOBULIN AND NGAL AS MARKERS FOR HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN NEONATES

By

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ABSTRACT

Background: *Hypoxic-ischemic encephalopathy (HIE) can develop due to perinatal asphyxia, and the brain is considered a highly susceptible organ. During the first 72 hours after a quick assessment, competent patients need neuroprotective medications to prevent brain harm. Consequently, the importance of investigating rapid, easily detected markers to help in early diagnosis with subsequent better prognosis.*

Objective: *We intended to measure and compare the usefulness of urinary neutrophil gelatinase-associated lipocalin (NGAL), Beta 2-microglobulin (β2M), and uric acid creatinine ratio (U/Cr) as diagnostic and prognostic markers in HIE.*

Methods: *This is a case-control study that was carried out during the period from September 2018 to September 2020 at the NICU of Menofia University Hospitals on 200 neonates who were selected by simple random method. Out of them, 100 patients had perinatal asphyxia complicated with HIE (cases) according to The American Academy Of Pediatrics and American College Of Obstetrics And Gynecology and another 100 healthy neonates as (control). Routine investigations, EEG and MRI, were performed for all cases. Urinary NGAL, β2M, and U/Cr ratios were measured for all studied neonates at admission and again at 48hs for cases.*

Results: *Our study showed that urinary U/Cr ratio, β2M, and NGAL were significantly higher in neonates suffering from HIE than in controls (2.38 vs. 0.96, 2.73 vs. 1.37, and 42.5 vs. 13.5; all $p<0.001$). Also, they were highly elevated in non–survivors compared to survivors and at admission compared to after 48 hs in survivors, while the opposite was the case in non-survivors, all $p \leq 0.001$). Neonates classified as stage III Sarnat reported elevated levels of the tested markers compared to stage I and II. The*

diagnostic ROC curve analysis clarified that urinary NGAL had an excellent diagnostic presentation to detect HIE (AUC=.992, cuttoff= 22.50), while β2M had the best prognostic value (AUC=0.883, cuttoff=2.80) and uric acid /creatinine ratio was the best indicator of disease severity (AUC=0.90, cuttoff=2.20).

Conclusion: *Urinary U/Cr ratio, β2M and NGAL are promising, available, non-invasive, painless, and low-cost predictive and prognostic additional support in early diagnosis of HIE, with high sensitivity and specificity going to urinary NGAL for diagnosis, β2M for prognosis and uric acid /creatinine ratio for severity.*

Keywords: *Urinary β2M microglobulin, Neutrophil Gelatinase Associated Lipocalin, uric acid/creatinine ratio, hypoxic-ischemic encephalopathy, Urinary Markers.*

INTRODUCTION

Perinatal asphyxia is a medical disorder caused by oxygen deprivation occurring at birth. Four million neonates suffer from perinatal asphyxia per year, leading to the death of one million **(Halloran et al., 2009)**. Asphyxia during pregnancy or delivery may result from several risk factors and as a result, multi-organs may become dysfunctional and cardiac output may be redistributed to preserve cardiac, cerebral, and adrenal perfusion while possibly compromising gastrointestinal, renal, and skin perfusions **(Choudhary et al., 2017)**.

Some of the asphyxiated babies may develop hypoxic-ischemic encephalopathy (HIE). 20-25% of asphyxiated babies die from severe HIE **(Riljak et al., 2016)**. About 25% of babies who survive severe HIE develop long-lasting neuropsychological hindrances such as epilepsy, and cerebral palsy, with the presence or absence of related mental retardation learning difficulties **(Bhatti et al., 2014)**.

Several biomarkers had been evaluated for neonates with birth asphyxia as some brain proteins (protein S-100, S-100B, and S-100A), neuron-specific enolase, and Creatine Kinase BB. These markers were elevated in neonates with birth asphyxia but still carry the idea of invasiveness **(Nagdyman et al., 2001)**.

Perinatal asphyxia leads to tissue hypoxia and converts xanthine to uric acid. A high urinary uric acid/ creatinine ratio (U/Cr) was observed in neonates with perinatal asphyxia **(Choudhary et al., 2017)**.

Beta-2 microglobulin is a low molecular weight protein reabsorbed by the kidney tubules. It can detect renal impairment caused by asphyxia within 48 h. Non-renal etiologies and pathologies associated with renal illness impact higher blood β2M

levels in non-black races and higher urinary excretion of the protein. It is secreted into the body fluids during normal cell turnover; hence pathologies with rapid cell turnover are linked with greater serum β2M levels **(Argyropoulos et al., 2017)**.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 24 kDa glycoprotein initially filtered from a kidney cell culture of murine contaminated with Simian virus 40. At the early phase of ischemia-induced acute kidney injury (AKI), NGAL levels are increased in serum, urine after ischemic kidney injury and plasma **(Essajee et al., 2015)**. NGAL has been identified as a biomarker in various non-renal diseases, including cerebral tumor, inflammatory bowel disease, and pre-eclampsia. NGAL also influences the rise and existence of specific cancerous cells, including hepatocellular carcinoma and thyroid cancer **(D'Anna et al., 2008)**.

Aims of the Work

To measure and compare the usefulness of urinary neutrophil gelatinase-associated lipocalin (NGAL), Beta 2-microglobulin (β2M), and uric acid creatinine ratio (U/Cr) as diagnostic and prognostic markers in neonates with HIE.

Ethical considerations:

1. An informed consent was taken from all parents of recruited neonates before getting involved in the study.
2. Confidentiality of all data was ensured.
3. The study was done after approval of ethical committees of pediatrics department &faculty of medicine of Menofia University.
4. The authors declared no potential conflicts of interest with respect to the research, authorship and /or publication of this article.
5. Financial disclosure/ Funding: The authors received no financial support for the research, authorship, and/or pbblication of this article.

Sample size:

The sample size was calculated using the following formula:

Where:

$$n = 2\left[\frac{\left(Z_{\alpha/2} + Z_{\beta}\right) * \sigma}{\mu_1 - \mu_2}\right]^2$$

n = sample size

$Z_{\alpha/2}$ = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail)

Z_β = 0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%)

σ = the estimate of the standard deviation of neonates with D-dimer level in the lowest quartile = 1.47

μ_1 = mean of neonates with D-dimer level in the lowest quartile was = 27.9

μ_2 = mean in of neonates with D-dimer level in the other quartiles follow up = 9.2

So, by calculation, the sample size required to conduct this study was 200 neonates in total.

PATIENTS AND METHODS

This is a case-control study that was conducted on 200 full-term and preterm neonates recruited by simple random method from the Neonatal Intensive Care Unit and the outpatient well baby clinic of Menofia University Hospitals in the period from September 2018 to September 2020.

Inclusion criteria:

The cases group: included 100 newborn diagnosed as perinatal asphyxia complicated with hypoxic-ischemic encephalopathy. Perinatal asphyxia was diagnosed according to the criteria of the American Academy of Pediatrics (AAP) and American College of obstetrics and gynecology (ACOG). Criteria of diagnosis include one or more of the following:

- Apgar score at 5 min and 10 min: less than 5
- Fetal umbilical artery pH: less than 7.0
- Arterial base deficit equal to or more than 12 mmol/L
- Acute brain damage can be seen in neuroimaging
- Presence of multisystemic organ failure is consistent with HIE (e.g., hepatic injury, hematological changes and renal failure) **(American Academy of Pediatrics, 2014)**.

Hypoxic-ischemic encephalopathy was further categorized into mild, moderate and severe according to the clinical Sarnat Staging System (Sarnat and Sarnat, 1976).

The control group: included 100 healthy age, sex-matched, and non-hospitalized neonates with no pathological findings, recorded in their physical examinations and recruited from Menofia University Hospital outpatient well-baby follow-up clinic.

Exclusion criteria:

We excluded neonates with:

1. Low Apgar score related to administered anesthesia.

2. Apparent congenital anomalies.
3. Significant illnesses such as pathological jaundice, severe infection or suspected inborn errors of metabolism.
4. Primary glomerular diseases such as nephritis.
5. Whose mothers' received nephrotoxic drugs such as aminoglycosides?

- **Study Process and Evaluations:**

Studied neonates in both groups were subjected to the following:

1. Full history taking (prenatal, natal and postnatal).

2. Complete clinical and neurological examination according to **(Yang et al., 2004)**.

3. Classification of cases according to Sarnat and Sarnat staging system to assess the degree of HIE.

4. Investigations:

Routine investigations including:

a. Complete blood count (CBC) by Coulter Miami USA (1996). **b.** C- reactive protein (CRP) by latex **(Wood and Maccarty, 1951) c.** Arterial blood gases (ABG) determination at the first day after birth, assayed on blood gas analyzer; Nova Fox (Altera board version Nova Biomedical: Waltham, MA 02454, USA). **d.** Blood chemistry: blood glucose, kidney function tests, liver function tests and serum electrolytes; assayed on chemistry analyzer (Cobas c111, Roche).

- **Specific investigations including:**

Measurement of urinary levels of uric acid, creatinine, β2M and NGAL. All investigations were done in accordance with the manufacturer's instructions, and were performed in the central laboratory of Menofia University Hospital.

6. Imaging and electrophysiological studies in the form of MRI and EEG for cases only.

Biochemical analysis:

The specific laboratory work was conducted in the clinical pathology department, Faculty of Medicine, Menoufia University. Two urine samples (3ml) were collected non-invasively by urine collecting bag at admission and after 48 hours. Samples were separated into three partitions (each 1ml). The first partition was centrifuged at 2000-3000 rounds per minute for 20 minutes. Then the supernatants were collected

carefully. When sediments occurred during storage, it was centrifuged again and stored at -20°C until assessment. After that, NGAL concentration was measured by utilizing a commercially available ELISA kit (Bioassay technology laboratory, china). The measurement range for this assay was 10ng/ml to3000ng/ml.

The second partition was stored at -20 °C until assessment. Then samples were diluted at 1:100 with N diluent. β2M was measured in the diluted samples used within 4 hours.

The third partition was utilized to assess urine uric acid using the modified Uricase technique and urinary creatinine using the modified kinetic method. Jaffe's thoughts on Dade Behring's dimension extend as well as an auto analyzer. For uric acid and creatinine, the intra and inter-assay coefficients of variance were 2.2% and 3.5%, respectively.

Statistical analysis:

The Statistical Package for Social Science (SPSS) version 21 was used for all analyses (SPSS Inc., Chicago, Illinois, USA).

1. The qualitative data were provided in frequencies and percentages
2. The chi-squared test was applied to analyze the connection among two variables or more.
3. The Fischer's exact test was utilized if the predicted cell count of one part of the instances was five or less.
4. The Student t-test and Mann-Whitney U test were employed to compare quantitative variables among two groups for parametric and non-parametric data.
5. The receiver operator characteristics (ROC) analysis was applied to evaluate biomarker performance, with maximum accuracy points for sensitivity and specificity.

When the p-value was less than 0.05, a significant difference was considered.

RESULTS

Table (1): Demographic and clinical characteristics of studied neonates

	Cases (n=100)	Controls (n=100)	P value
Gestational age (weeks)	37.97±1.45	38.30±1.38	0.100
Age (days)	2.20±1.63	2.33±1.54	0.78
Sex			
Male	75 (75%)	62 (62%)	0.067
Female	25 (25%)	38 (38%)	
Mode of delivery			
Vaginal	25 (25%)	50 (50%)	0.001[a]
C.S	75 (75%)	50 (50%)	
Apgar score after 1 minute	2.55±1.50	8.90±1.14	0.001[a]
Apgar score after 5 minutes	4.70±1.42	9.60±0.66	0.001[a]
Resuscitation Procedure			
Yes	55 (55%)	-	-
No	45 (45%)		
HIE Staging			
Stage 1	30 (30%)	-	-
Stage 2	50 (50%)		
Stage 3	20 (20%)		
Mechanical ventilation			
Yes	65 (65%)	-	-
No	35 (35%)		
Weight (gm)	2.98±0.42	3.12±0.66	0.075
Consanguinity			
Yes	25 (25%)	28 (28%)	0.749
No	75 (75%)	72 (72%)	
Non-survivors	20 (20%)	-	-

HIE: Hypoxic-Ischemic Encephalopathy. ap<0.05 considered significant

Table 1: shows that there were high significant differences between the studied groups regarding mode of delivery, Apgar scores at 1st and at 5th minutes. While non-significant differences were elicited regarding other data.

Table (2): Laboratory results of the studied neonates

Variables	Group I No.=100	Group II No=100	Test of sig.	P value
Hb (g/dl)				
Mean ± SD	14.22±2.85	13.20±1.17	t	
Range	10.2-20.7	12-15		0.001
Median	13.60	13	3.31	S
WBC(cell/mm^3)				
Mean ± SD	11.91±4.02	8.77±2.02	t	
Range	3.5-33.4	4.8-12		≤0.001
Median	9.55	7.90	5.44	S
Platelets(cell/mm^3)				
Mean ± SD	213.9±80.90	256.60±62.36	U	
Range	85-389	180-370	4.95	≤0.001
Median	195	233		HS
PH				
Mean ± SD	7.01±0.06	7.38±0.05	t	
Range	6.89-7.21	7.32-7.42	11.65	≤0.001
Median	7.09	7.37		HS
PCO2				
Mean ± SD	49.50±9.53	40.07±4.61	t	
Range	32 – 81	32 – 48		0.003
Median	62	40	3.3	S
HCO3				
Mean ± SD	15.04±4.04	23.90±2.22	t	
Range	7-21	22-26		≤0.001
Median	17.50	23.50	8.87	HS
Base deficit			U	
Mean ± SD	-8.49±3.14	1.56±1.28		≤0.001
Range	-11-(-3)	-2-2	9.86	HS
Median	-8.35	0.5		
Urea	57.53±22.03		U	
Mean ± SD	28-116	27.93±7.16		≤0.001
Range	64	18-40	5.83	HS
Median		20		
Creatinine				
Mean ± SD	1.08±0.48	0.68±0.17	**U**	≤0.001
Range	0.50-2	0.40-0.90	5.73	HS
Median	0.80	0.70		
Na$^+$				
Mean ± SD	139.45±8.06	139.80±3.78	**t**	0.695
Range	129-158	135-147	**0.393**	NS
Median	136.50	139.5		
K$^+$			t	
Mean ± SD	3.44±0.64	3.78±0.64		≤0.001
Range	2.50-4.50	3-5	3.67	HS
Median	3.50	3.80		

Ca^{++}			t	
Mean ± SD	9.30±0.77	9.37±0.86		0.568
Range	8-10.5	8-11	0.57	NS
Median	9.30	9.15		
ALT				
Mean ± SD	26.55±27.9	6.10±2.2	U	
Range	5-66	3-10		≤0.001
Median	29	7	8.36	HS
AST			U	
Mean ± SD	39.8±31.10	9.60±2.21		≤0.001
Range	12-110	7-15	11.8	HS
Median	45	11		

NS=non-significant, S=significant, HS=High significant, U=Mann-whiteny test, t=students t test

Table 2: shows that there were statistically significant differences between the studied groups regarding all the measured laboratory findings except for Na^{+} and Ca^{++} levels.

Table (3): Values of the UA/Cr ratio, B2M, and NGAL markers in studied groups

	Cases (n=100)	**Control (n=80)**	**p-value**
UA/Cr ratio	2.38±0.471	0.96±0.186	<0.001[a]
B2M (mg/l)	2.73±0.792	1.37±0.437	<0.001[a]
NGAL (ng/ml)	71.25±66.37	13.40±3.87	<0.001[a]

UA/Cr ratio, Uric acid/ creatinine ratio; B2M, B2-microglobuline; NGAL, Neutrophil gelatinase associated lipocalin. Ap <0.05 considered significant.

Table 3: shows that there were statistically significant differences in urinary UA/Cr ratio, B2 microglobuline and NGAL between the studied groups, being higher in cases than controls.

Table (4): Correlation between markers and other studied parameters in cases

	UA/Cr ratio		B2M (mg/l)		NGAL (ng/ml)	
	r	P-value	R	P-value	r	P-value
Apgar score at 1 minute	-0.453	<0.001[a]	-0.777	<0.001[a]	-0.482	<0.001[a]
Apgar score at 5 minutes	-0.486	<0.001[a]	-0.792	<0.001[a]	-0.439	<0.001[a]
Duration of incubation	0.655	<0.001[a]	0.385	0.002[a]	-0.341	0.005[a]
O2 saturation	-0.019	0.854	-0.472	<0.001[a]	-0.229	0.001[a]
Gestational age (weeks)	-0.219	0.029[a]	-0.310	<0.001[a]	-0.166	0.101
Weight (cm)	-0.296	0.003[a]	-0.464	<0.001[a]	-0.046	0.505
Height (cm)	-0.117	0.244	-0.410	<0.001[a]	-0.123	0.082
Respiratory rate	-0.300	0.002[a]	0.384	<0.001[a]	0.368	<0.001[a]
Heart rate	-0.071	0.484	-0.241	0.001[a]	-0.092	0.197
Systolic BP (mm/Hg)	-0.704	<0.001[a]	-0.676	<0.001[a]	-0.08	0.229
Diastolic BP (mm/Hg)	-0.576	<0.001[a]	-0.560	<0.001[a]	-0.008	0.927
Hb (g/dl)	0.252	0.011[a]	0.320	<0.00[a]	0.446	<0.001[a]
WBC (cell/mm^3)	-0.153	0.151	0.298	<0.001[a]	0.683	<0.001[a]
Platelets (cell/mm^3)	-0.465	<0.001[a]	-0.443	<0.001[a]	-0.102	0.152
PH	-0.348	<0.001[a]	-0.695	<0.001[a]	-0.324	<0.001[a]
PCO2	0.65	<0.001[a]	0.64	<0.001[a]	0.68	<0.001[a]
HCO3	-0.220	0.028[a]	-0.530	<0.001[a]	-0.269	<0.001[a]
Base deficit	0.330	0.001[a]	0.641	<0.001[a]	0.257	<0.001[a]
Creatinine (mg/dl)	0.028	0.698	0.130	0.067	0.132	0.065
Urea (mg/dl)	0.074	0.295	0.129	0.069	0.033	0.647
Potassium	-0.083	0.410	0.632	<0.001[a]	0.062	0.382
Sodium	-0.248	0.015[a]	0.679	<0.001[a]	0.071	0.320
ALT (U/L)	0.642	<0.001[a]	-0.140	0.048[a]	-0.037	0.601
AST (U/L)	0.725	<0.001[a]	-0.179	0.011[a]	-0.135	0.056
Calcium	-0.465	<0.001[a]	-0.064	0.395	-0.024	0.753
B2M (mg/l)	0.708	<0.001[a]	-	-	-	-
NGAL (ng/ml)	0.227	0.001[a]	0.504	<0.001[a]	-	-

BP, Blood pressure; WBCs, White blood cells; HB, Hemoglobin; PCO2, Partial pressure of carbon dioxide; HCO3, Bicarbonate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UA/Cr ratio, Uric acid/ creatinine ratio; B2M, B2-microglobuline NGAL, Neutrophil gelatinase associated lipocalin; ap<0.05 considered significant.

Table 4: shows that UA/Cr and β2M ratio were significantly correlated with most of the studied biological parameters. NGAL was significantly correlated with respiratory rate, Hb, WBCs, blood gases parameters (PH, PCO2, HCO3, Base deficit, and O2 saturation), and creatinine .There was a significant strong positive correlation between β2M and both UA/Cr ratios and NGAL. UA/Cr had a weak significant positive correlation with NGAL.

Table (5): Values of UA/Cr ratio, B2M, and NGAL`in relation to the outcome of HIE cases

		Non-survivors (n=20)	Survivors (n=80)	p-value
UA/Cr ratio		2.05±0.878	1.53±0.44	0.019[a]
B2M (mg/l)		2.90±0.692	2.51±0.656	≤0.001[a]
NGAL (ng/ml)		76.25±60.2	52.60±33.1	0.017[a]
		At Admission	**After 48 Hours**	**p-value**
Non-survivors	**UA/Cr ratio**	2.05±0.878	2.65±0.158	≤0.001[a]
	B2M (mg/l)	2.90±0.692	3.60±0.663	≤0.001[a]
	NGAL (ng/ml)	76.25±60.2	80.25±13.98	≤0.001[a]
Survivors	**UA/Cr ratio**	1.53±0.44	1.50±0.667	≤0.001[a]
	B2M (mg/l)	2.51±0.656	1.97±0.572	≤0.001[a]
	NGAL (ng/ml)	52.60±33.1	32.93±8.92	≤0.001[a]

UA/Cr ratio, Uric acid/ creatinine ratio; B2M, B2-microglobuline; NGAL, Neutrophil gelatinase associated lipocalin. ap<0.05 considered significant.

Table 5: shows that there were statistically significant differences in urinary UA/Cr ratio, B2 microglobuline and NGAL between Non-survivors and Survivors, being higher in non-survivors.

In survivors group there were statistically significant increase in urinary uric acid creatinine ratio, B2 microglobuline and NGAL at admission than after 48 hour. While in non survivors group the urinary markers increased after 48h than at admission.

Table (6): Results of UA/Cr ratio, B2M, and NGAL of neonates with asphyxia in relation to Sarnat stages

	Stage I	Stage II	Stage III	P-value
UA/Cr ratio	1.39±0.239	1.62±0.509	2.05±0.878	P = 0.05 P1 = 0.07 P2 ≤ 0.001[a] P3 = 0.004[a]
B2M (mg/l)	2.46±0.672	2.59±0.667	3.47±0.828	P ≤0.001[a] P1 = 0.449 P2 ≤ 0.001[a] P3 ≤ 0.001[a]
NGAL (ng/ml)	35±7.21	75.10±60.6	89.0±86.6	P = 0.005[a] P1 = 0.351 P2 = 0.004[a] P3 = 0.020[a]

UA/Cr ratio, Uric acid/ creatinine ratio; B2M, B2-microglobuline; NGAL, Neutrophil gelatinase associated lipocalin. ap<0.05 considered significant.
P1: Stage I Vs Stage II - P2: Stage I Vs Stage III - P3: Stage II vs Stage III

Table 6 shows that there were statistically significant differences in urinary uric acid creatinine ratio, B2 microglobuline and NGAL of the studied neonates with asphyxia in relation to sarnat stages being higher in stage 3 in comparison to 1 and 2.

Table (7): Validity of markers for prediction and assessing prognosis of perinatal asphyxia

	Asphyxia prediction			Asphyxia mortality			Asphyxia severity		
	UA/Cr ratio	B2M	NGAL	UA/Cr ratio	B2M	NGAL	UA/Cr ratio	B2M	NGAL
AUC	0.90	0.962	0.992	0.641	0.883	0.430	0.90	0.78	0.67
Cutoff point	1.035	1.77	22.50	1.25	2.80	42.50	2.20	2.80	39.50
Sensitivity	90%	95%	95%	75%	100%	50%	95%	75%	75%
Specificity	70%	80%	100%	31.2%	75%	50%	60%	68.7%	39.7%
NPV%	88%	94%	95%	73%	100%	50%	75%	90%	29%
PPV%	75%	83%	100%	70%	80%	50%	90%	39%	38%

UA/Cr ratio, Uric acid/ creatinine ratio; B2M, B2-microglobuline NGAL, Neutrophil gelatinase associated lipocalin; AUC, Area under curve; NPV%, Negative predictive value; PPV%, Positive predictive value.

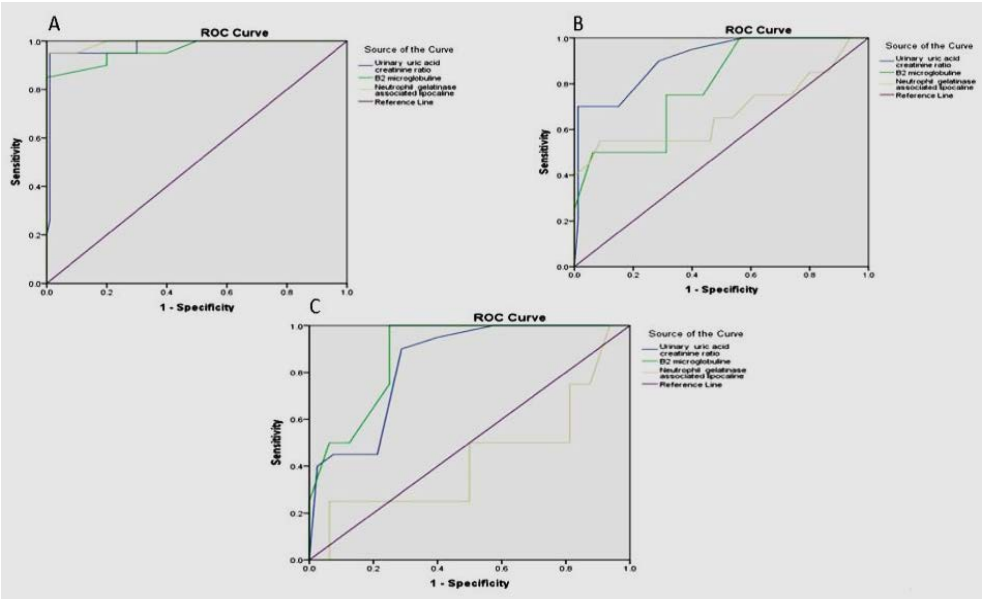

Figure (1): Receiver-operating characteristic (ROC) curve of UA/Cr ratio, B2M, and NGAL for perinatal asphyxia (a) diagnosis, (b) mortality prediction, and (c) severity prediction.

Table 7 & Fig. 1: shows that UA/Cr, β2M, and NGAL had a high discrimination power to detect neonatal HIE (AUC>0.9) at cut-off points (1.035, 1.77, and 22.50, respectively) with the best sensitivity, specificity, PPV and NPV going to urinary NGAL in diagnosis, figure 1a. Regarding mortality prediction, β2M (AUC=0.883, cutoff=2.80) is more efficient than UA/Cr and NGAL (AUC= 0.641, cutoff=1.25; and AUC= 0.430, cutoff=42.50, respectively), figure 1b. The differentiation power of UA/Cr ratio (AUC=0.9, cuttof= 2.2,) was higher than β2M, and NGAL (AUC= 0.78, cuttoff=2.8 and AUC=0.67, cutoff=39.5) to detect severe cases, figure 1c.

DISCUSSION

Asphyxia is still a serious disorder that causes death and morbidity. The neurological manifestation of systemic hypoxia in newborns is HIE. It is the most common cause of seizures and handicaps in newborns **(Bahubali et al., 2013)**. This study examined the diagnostic and prognostic performance of UA/Cr ratio, β2M, and NGAL in neonates with perinatal asphyxia complicated with HIE.

The mean uric acid/creatinine ratio in asphyxiated neonates of our study was increased than in controls (2.38 vs. 0.96, p<0.001). The same findings were shown by **(Choudhary et al., 2017)** (3.1 vs. 0.96); p<0.01. This can be explained by that anaerobic glycolysis occurs in newborns with perinatal asphyxia as a result of the hypoxia-induced failure of brain oxidative metabolism, resulting in just 2 molecules of Adenosine Triphosphate (ATP) being produced as opposed to 32 molecules of ATP being produced during aerobic circumstances **(Basu et al., 2008)**. Lack of ATP and accelerated cellular death result in a buildup of Adenosine Monophosphate (AMP) and Adenosine Diphosphate (ADP), which are then catabolized to their components adenosine, inosine, and hypoxanthine. In the presence of xanthine oxidase, ongoing reduced oxygenation of the tissue and subsequent reperfusion damage will lead to hypoxanthine being converted to xanthine and uric acid. As a result, uric acid production will rise and enter the bloodstream from injured tissues. This uric acid is subsequently expelled in the urine, where it is easily identified **(Patel et al., 2017)**.

Compared to the control group, the mean β2M was significantly

increased in the asphyxiated neonates (2.73 vs. 1.37, p<0.001), indicating its potential to detect renal impairment and diagnose the occurrence of HIE. **Banerjee et al., 2013** and **El-Gendy et al., 2014** presented the same results (4.1 vs. 1.35, 12.5 vs. 0.2); all p<0.001 (24, 25). Many cells express beta 2 microglobulin at a steady level, and its increased levels may be due to renal impairment or excessive production. In the presence of IFN during infilammatory processes, β2M formation would be augmented which in turn causes regulation of hormone/growth factor expression, and coordination of the interaction among cytokines and their receptors **(Li et al., 2016)**. The pathogenesis of prenatal asphyxia is most likely linked to inflammatory mediators like cytokines. According to one research, cytokines induce brain damage by directly injuring the white matter, compromising the germinal matrix endothelium, causing brain bleeding, and inducing inflammatory responses in microglia and astrocytes. Hence increased levels of β2M microglobulin in HIE could be interpreted by its role in the cytokines pathways **(Dammann et al., 1997)**.

Our study reprted that mean NGAL is significantly upsurged in asphyxiated neonates compared to the control group (42.5 vs. 13.5, p<0.001). These findings are consistent with those of **(Chandrashekar et al., 2016)** who revealed a highly significant rise in the serum NGAL levels in cases group in comparison to controls. Elevations could be caused by an injury to the endothelial cells of vessels everywhere in the body, plus many inflammatory stimuli, such as lipopolysaccharides and IL-1β, that can stimulate lipocalin-2 expression and secretion in these cells **(Jayaraman et al., 2005)**. Our work clarified that the mean urinary U/Cr, β2M, and NGAL differed significantly between survivors and non-survivors, being lower in survivors. The lower levels of markers in survivors could be explained when the majority of survivors were presented in stages I and II of Sarnat staging, and in our results, we found that the estimated markers were decreased in these two stages than in stage III. On evaluating the studied markers at admission and again at 48h, we reported a decrease in their urinary levels in survivors after 48hs than at admission levels, in contrast to non-survivors who encountered an increase in the levels of the

markers after 48h of admission, emphasizing the relationship between the studied markers and the severity of the disease. Regarding Sarnat staging for HIE, the current study reported that UA/Cr ratios were significantly increased in neonates with severe HIE when coming in comparison with those having mild and moderate HIE. This agrees with **(Choudhary et al., 2017)** who demonstrated a positive correlation between the severity of HIE and the urinary UA/Cr ratio. Furthermore our work detected an increase in the urinary level of β-2 microglobulin with respect to the encephalopathy degree, this agrees with results of **(Banerjee et al., 2013)**. The mean urinary level of NGAL at admission was significantly increased in HIE stage III than stage II and stage I. In line with our results, are **(El Raggal et al., 2013** and **Chandrashekar et al., 2016)**.

On one hand our results showed a highly significant negative correlation between urinary UA/Cr and Apgar score at 1 and 5 minutes, arterial pH, HCO3, gestational age, weight, respiratory rate, systolic BP, diastolic BP, platelets, and Na+. On the other hand, a highly significant positive correlation was observed among urinary UA/Cr ratio and PCO2, Duration of incubation, Hb, base deficit, ALT, AST, β2M, and NGAL, This agrees with results reported by **(Bhongir et al., 2015)**.

There were statistically significant negative correlations between β2M and Apgar score at 1 and 5 minutes, oxygen saturation, gestational age, weight, height, heart rate, systolic BP, diastolic BP, platelets, PH, HCO3, K+, and Na+. This is in accordance with previous studies **(Li et al., 2016)**. Urinary NGAL had a statistically significant positive correlation with respiratory rate, Hb, WBCs, PCo2 and base deficit. At the same time, there were statistically significant negative correlations between it and Apgar score at 1st and 5th mins, duration of incubation, O2 saturation, PH and HCO3.

According to our results, the sensitivity, specificity, PPV and NPV of urinary U/Cr in predicting HIE at a cut-off value≥1.035 were 90, 70, 88 and 75%, respectively. These results are not so different from the results estimated by **(Choudhalry et al., 2017** and **Patel et al., 2017)**. Furthermore, the predictive value of β2M as a diagnostic marker for HIE showed a sensitivity of 95, specificity of (80%, NPV of 94%, and PPV of 83%. These results are in line with **(El-Gendy et al., 2014)**. In the

current work, the sensitivity and specificity of urinary NGAL in detecting HIE were 95% and 100%, respectively, with 100% PPV and 95% NPV. The findings described above are quite similar to those of a study of urinary NGAL conducted by **(Essajee et al., 2015)**.Our study reported the validity of urinary UA/Cr ratio, β2M and NGAL as a prognostic markers for perinatal asphyxia with the best results going to β2M as it reported 100% sensitivity, 75% specificity, 100% NPV, and 80% PPV.

CONCLUSION

Urinary UA/Cr ratio, β2M, and NGAL are available, invasive-free, pain-free and low-cost additional support with good predictive and prognostic values in HIE. Urinary NGAL has the best diagnostic potential, while β2M has the best prognostic one.

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دراسة البيتا 2 ميكروجلوبيولين و الليبوكالين المصاحب لانزيم الجيلاتيناز للخلايا البيضاء المحببة المتعادلة و نسبة حمض اليوريك البولي للكرياتينين في الاختناق الوليدي

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خلفيـــة: الاختنـــاق الوليـــدى هـــو حالـــة طبيـــة ناتجـــة عـــن حرمـــان حـــديثي الـــولادة مـــن الاكســـجين والـــذي يســـتمر طـــويلا اثنـــاء عمليـــة الـــولادة ليســـبب اذي جســـدى وفـــي معظـــم الاحيـــان للمـــخ ممـــا يـــؤدى الي اعتلال الدماغ الوليدى.

الاختنـــاق الوليـــدى يـــؤدى الـــي حـــالات مرضـــية ووفيـــات بنســـبة كبيـــرة مـــن حالـــة الـــي ســـت حـــالات مـــن كـــل الـــف مولـــود مكتمـــل الاشـــهر الجنينيـــة وتمثـــل ثالـــث اهـــم ســـبب فـــي وفيـــات حـــديثي الولادة.لـــذا فهنـــاك ضـــرورة ملحـــة للبحـــث عـــن طـــرق تشـــخيص سريعة و سهلة للمساعدة في التشخيص المبكر.

الهـــدف: قيـــاس ومقارنـــة نســـبة حمـــض اليوريـــك الـــي الكريـــاتينين, البيتـــا 2ميكروجلوبيـــولين والليبوكـــالين المصـــاحب لانـــزيم الجيلاتينيـــز للخلايـــا البيضـــاء المحببـــة المتعادلـــة فـــي حـــديثي الـــولادة الـــذين يعـــانون مـــن الاختنـــاق الوليـــدى مـــع اصـــحاء حـــديثي الـــولادة وتوضـــيح دورهـــم التشخيصـــي والتنبـــؤى عنـــد هـــؤلاء حـــديثي الولادة.

المرضـــي وطـــرق البحـــث: فـــي هـــذه الدراســـة تـــم دراســـة جميـــع حالات الاطفال المبتسرين المصابين بالاختناق الوليدى.

ويخضع المريض للفحوصات التالية:

- اخذ التاريخ المرضي للمريض.
- فحص جسدي كامل.
- الفحوصـــات المعمليـــة وتتضـــمن فحوصـــات روتينيـــة مثـــل (ســـي بـــي ســـي-وظـــائف كلـــي –وظـــائف كبـــد- الكترولايـــت) وفحوصـــات متخصصـــة (نســـبة حمـــض اليوريـــك البـــولي الـــي الكرياتينين، الليبوكالين، البيتا تو ميكروجلوبيولين).

هذه الدراسة شملت 200مولود يقسموا الي مجموعتان:

الاولـــي:100 مولـــود يعـــانون مـــن الاختنـــاق الوليـــدى البســـيط والمتوسط والشديد.

الثانية:100مولود لا يعانون من الاختناق الوليدى.

تـــم تجميـــع البـــول بواســـطة كـــيس جمـــع بـــول وتقـــاس هـــذه الواســـمات فـــي المواليـــد المكتملـــة النمـــو والغيـــر مكتملـــة النمـــو والـــذين يعـــانون مـــن الاختنـــاق الوليـــدى وتقـــارن بقياســـات المواليـــد الذين لا يعانون من الاختناق الوليدى

النتائج:

- هناك فرق كبير بين المجموعة المختنقة ومجموعات الضبط فيما يتعلق بأن جميع الدلالات كانت أعلى في الولدان المختنقين و ايضا في الذين توفوا عن الذين عاشوا.

- اليبوكالين المصاحب لانزيم الجيلاتيناز للخلايا البيضاء المحببة المتعادلة هو افضل الدلالات في التنبؤ بحدوث الاعتلال الدماغي المصاحب للاختناق الوليدي.

- البيتا 2 ميكروجلوبيولين هو اكثر الدلالات تنبؤا بالحالة النهائية للمريض.

الاستنتاج: البيتا 2 ميكروجلوبيولين و الليبوكالين المصاحب لانزيم الجيلاتيناز للخلايا البيضاء المحببة المتعادلة و نسبة حمض اليوريك البولي للكرياتينين في حديثي الولادة الذين يعانون من الاختناق الوليدى يمثلون إطار عمل إضافي يمكن الاستعانة بهم حيث انهم و بالاخص البيتا 2 ميكروجلوبيولين و الليبوكالين المصاحب لانزيم الجيلاتيناز للخلايا البيضاء المحببة المتعادلة لهم قيمة تنبؤية جيدة لتشخيص الاختناق و متابعة عواقبة في الفترة المحيطة بالولادة.

الكلمات الرئيسية: البيتا 2 ميكروجلوبيولين، الليبوكالين المصاحب لانزيم الجيلاتيناز للخلايا البيضاء المحببة المتعادلة، نسبة حمض اليوريك البولي للكرياتينين، حديثي الولادة، الاختناق الوليدى.