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Original article

Urinary protein markers of renal dysfunction in full-term newborns with disorders of early neonatal period

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Abstract

Background: Urinary proteins may help to understand the physiology and diagnose renal dysfunction in sick infants. The renal function in ill full-term newborns with disorders of early neonatal period by means of detecting specific protein biomarkers in the urine was studied.

Materials and methods: A prospective cohort study on 205 full-term newborns was performed including 55 healthy infants, 55 newborns with clinical signs of moderate disorders of early neonatal period, 50 newborns with severe neonatal disorders without acute kidney injury (AKI), and 45 newborns with both severe neonatal disorders and AKI. The urinary concentrations of total protein (UTPr), albumin (UAlb), immunoglobulin G (UIgG), α1-microglobulin (Uα1-MG), and β2-microglobulin (Uβ2-MG) were determined by means of laboratory tests.

Results: As compared to healthy newborns, full-term neonates with moderate disorders of early neonatal period developed dysfunction of the glomerular membrane (selective proteinuria with excessive excretion of UAlb), and proximal tubules (increased Uα1-MG). More severe neonatal disorders in term newborns are accompanied by selective proteinuria together with more pronounced tubular failure (excessive urinary excretion of Uα1-MG and Uβ2-MG). Formation of AKI in term infants is associated with complex disorders of all structural elements of the nephron, manifested by extremely high levels of total protein markers of glomerular (UTPr, UAlb and UIgG) and tubular (Uα1-MG and Uβ2-MG) dysfunction in urine.

Conclusions: The measurement of urinary protein biomarkers in sick full-term newborns with clinical signs of disorders of early neonatal period demonstrated renal dysfunction not only in infants with AKI. The biochemical changes found in critically ill newborns require timely diagnosis promoting the right choice of intensive therapy with the aim to prevent the development

of severe renal pathology and chronic renal failure in the future.

Keywords

Full-term newborn, renal dysfunction, acute kidney injury, urinary protein markers.

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Introduction

The risk of acute kidney injury (AKI) in hospitalized critically ill newborns without primary renal disease remains to be high in both term and premature infants [1]. The diagnosis of AKI in newborns is problematic with current methods that rely on two functional abnormalities: functional changes in serum creatinine (SCr) and urine output. They both are late consequences of injury and not predictive markers of the injury itself [2-4].

SCr is a late and non-specific marker of reduced glomerular filtration rate (GFR). It is insensitive to acute changes in kidney function. Neither the cause, location of renal disease (e.g. pre-renal versus intrinsic; affected renal tubule segment; nephrotoxic versus ischemic AKI) nor the extent of renal damage are adequately reflected by SCr concentration. Jaffe method of measuring SCr interferes with conditions such as hyperbilirubinemia, hypertriglyceridemia, hemolysis and ketone bodies in the blood. Also, SCr is influenced by several non-renal factors such as muscle mass, medications taken, diet and tubular secretion, thus causing inaccuracies in making the diagnosis of AKI. In fact, SCr assesses only the function of glomerular filtration and not tubular secretion or reabsorption [5-7].

In the last decade a significant progress has been made in identifying and validating new biomarkers for AKI [7, 8]. Increasing introduction of the so-called "omics" into clinical practice, especially metabolomics, seems to offer new attractive prospects for improving neonatal outcomes and management of kidney disease, especially in critically ill newborns [2].

Urinary proteins may help to understand the physiology and improve making the diagnosis of renal dysfunction in sick infants. We found no data in the literature concerning changes of the renal function in newborns with different degrees of disorders during their first week of life, with or without signs of AKI.

Objective

To study renal function in full-term newborns with disorders of early neonatal period by detecting specific protein biomarkers in the urine.

Materials and methods

A prospective cohort study of 205 full-term neonates admitted between January 2014 and December 2016 has been performed. They were classified on the basis of the neonatal Therapeutic Intervention Scoring System (nTISS) [9]. The first group (group I) included 55 healthy newborns who had nTISS score zero. Group II included 55 neonates with clinical signs of moderate disorders of early neonatal period who had nTISS score in the range 1-9. Group III included 95 neonates who had clinical symptoms of severe disorders in their first postnatal week and had nTISS score 10 or higher. This group included 50 critically ill newborns without AKI (group IIIA) and 45 critically ill newborns with AKI (group IIIB).

The exclusion criteria of the study were birth weight ≤ 2,500 g, early neonatal sepsis and major congenital anomalies of the kidneys and urinary tract.

The following clinical data were picked out from the patients' medical records: signs of disorders of early neonatal period, factors of therapeutic intervention and signs of renal dysfunction.

The definition of AKI proposed by Jetton and Askenazi based on the Acute Kidney Injury Network (AKIN) classification was used: increase of SCr by 0.3 mg/dl (26.5 μmol/l) or by 150-200% from the previous value and/or level of urine output less than 0.5 ml/kg/h for 6 to 12 hours [6].

Urine and blood samples were collected on the 3rd day and in cases of anuria/oliguria after restoration of diuresis. Creatinine levels in serum

(SCr) and urine (UCr) were measured using enzymatic method (Creatinine 10493986, Siemens Healthcare Diagnostics, USA) by ADVIA®KC 1800/2400 Chemistry System (Siemens Healthcare Diagnostics, USA). Urea levels in serum (SUr) and urea (UUr) were measured using kinetic method (Urea 10494054, Siemens Healthcare Diagnostics, USA) by ADVIA®KC 1800/2400 Chemistry System (Siemens Healthcare Diagnostics, USA). The urinary concentration of total protein (UTPr) was measured using protein dye-binding method (UPRO_2 10494059, Siemens Healthcare Diagnostics, USA) by ADVIA® 1800 Chemistry System (Siemens Healthcare Diagnostics, USA). Urinary albumin (UAlb) was measured using immunoturbidimetric method (μALB 10494053, Siemens Healthcare Diagnostics, USA) by ADVIA® 1800 Chemistry System (Siemens Healthcare Diagnostics, USA). The urinary concentration of immunoglobulin G (UIgG), α1-microglobulin (Uα1-MG) and β2-microglobulin (Uβ2-MG) were measured using immunonephelometric method (Antiserum Human IgG, LATEX α1-microglobulin, LATEX β2-microglobulin, Siemens Healthcare Diagnostics, USA) by BN ProSpec System (Siemens, USA). All the tests kits were manufactured by the laboratory Gemeinschaftslabor Cottbus (Germany).

GFR was calculated on the basis of Schwartz's formula with modification by Bagdasarova: GFR (ml/min/1.73 m^2) = k · d (cm) / SCr (μmol/l) · 0.0113, where k = 0.45 for term neonates, d is the body length (cm), and SCr is the creatinine level in serum (μmol/l) [10].

The study was approved by the research ethics committee of Bukovinian State Medical University. Informed written consent was obtained from parents prior to enrollment of their babies into the study. All studies were conducted in compliance with the basic provisions of the Good Clinical Practice (1996), Council of Europe Convention on Human Rights and Biomedicine (1997), Helsinki Declaration of the World Medical Association on Ethical Principles for Medical Research (1964-2008).

Statistical analysis was performed by means of the software Statistica 7.0 (StatSoftInc., USA). The results of each group are expressed as mean (M) ± standard deviation (SD) and 95% confidence interval (95% CI) for symmetric distributions. The normality of data distribution was tested using the Shapiro-Wilk test for sample size ≥ 30. To compare continuous variables, parametric tests (independent t test) were used. Fisher's exact test was used to compare categorical variables. The difference of the parameters was considered to be statistically significant with $p < 0.05$.

Results

205 full-term neonates were enrolled in the study, including 55 healthy newborns (group I), 55 newborns with clinical signs of moderate degree of disorders in early neonatal period (group II), 50 newborns with severe neonatal disorders without AKI (group IIIA) and 45 newborns with severe neonatal disorders with AKI (group IIIB). Following recommendations [6], AKI was defined in the group IIIB in 15 neonates (33.3%) on the basis of increased SCr level, in 20 neonates (44.4%) by urine output, and in 10 neonates (22.2%) by combination of the two criteria.

Summaries of the epidemiological data are presented in **Tab. 1**. No statistical differences exist in gestational age, body mass and sex among the groups.

The results of our studies demonstrated that all infants with disorders of early neonatal period had complex somatic and neurological pathologies that could lead to the development of renal dysfunction.

Table 1. Neonatal epidemiological data.

	Group I (n = 55)	Group II (n = 55)	Group III (n = 95)	
			Group IIIA (n = 50)	Group IIIB (n = 45)
Gestational age, weeks, M ± SD (95% CI)	39.1 ± 1.15 (38.9-39.3)	39.0 ± 1.21 (38.8-39.1)	38.9 ± 1.34 (38.6-39.2)	39.0 ± 1.39 (38.5-39.5)
Body mass, g, M ± SD (95% CI)	3,396.1 ± 437.43 (3,279.8-3,514.1)	3,296.8 ± 424.02 (3,186.3-3,407.3)	3,374.9 ± 521.4 (3,228.3-3,521.5)	3,459.5 ± 494.17 (3,311.1-3,608.0)
Boys, n (%)	32 (58.2)	30 (54.5)	28 (56.0)	28 (62.2)
Girls, n (%)	23 (41.8)	25 (45.5)	22 (44.0)	17 (37.8)

The healthy newborns did not have any problems in their early neonatal period.

Among the 55 patients in group II, 28 (50.9%) had mild hypoxic-ischemic encephalopathy (HIE), 8 (14.5%) had moderate HIE, 10 (18.2%) had haemolytic disease of the newborn, 5 (9.1%) had moderate asphyxia, and 4 (7.3%) had diabetic fetopathy. 21 newborns (38.2%) had clinical signs of mild respiratory failure, 8 (14.5%) had moderate respiratory failure and 5 (9.1%) had cardiovascular failure.

As compared to group II, the critically ill neonates from group IIIA had more serious disorders in the first week of their life. 15 (30.0%) newborns had signs of moderate asphyxia, 3 (6.0%) had severe asphyxia, 8 (16.0%) had meconium aspiration, 10 (20.0%) had moderate HIE, and 14 (28.0%) had severe HIE. Most newborns in this group had clinical signs of multiple organ dysfunction syndrome (MODS): all of them (50 [100%]) had severe respiratory failure ($p_{IIIA\text{-}II} < 0.05$), 12 (24.0%) had cardiovascular failure ($p_{IIIA\text{-}II} < 0.05$), 9 (18.0%) had hemorrhagic syndrome ($p_{IIIA\text{-}II} < 0.05$), 6 (12.0%) had seizures ($p_{IIIA\text{-}II} < 0.05$), 5 (10.0%) had anemia, and 3 (6.0%) had necrotizing enterocolitis.

45 neonates had AKI (group IIIB). Certain association between AKI and severe asphyxia, cardiovascular failure, and necrotizing enterocolitis was found. In this group, 8 (17.8%) newborns had signs of moderate asphyxia ($p_{IIIB\text{-}II} < 0.05$), 12 (26.7%) had severe asphyxia ($p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$), 9 (20.0%) had meconium aspiration ($p_{IIIB\text{-}II} < 0.05$), 6 (13.3%) had moderate HIE, and 10 (22.2%) had severe HIE ($p_{IIIB\text{-}II} < 0.05$). MODS occurred in all critically ill full-term neonates with AKI. Severe respiratory failure was found in all 45 (100.0%) patients in group IIIB, cardiovascular failure in 31 (68.9%; $p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$), hemorrhagic syndrome in 9 (20.0%; $p_{IIIB\text{-}II} < 0.05$), seizures in 9 (20.0%; $p_{IIIB\text{-}II} < 0.05$), and anaemia in 8 (17.8%). Eight (17.8%) neonates with AKI developed necrotizing enterocolitis ($p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$).

In addition, neonates from group IIIB had significantly more therapeutic interventions and drug loading than infants in groups IIIA and II (**Tab. 2**). This produced an adverse effect on the renal function. Certain association between AKI and frequency of administration of inotropic drugs, especially high doses of dobutamine and dopamine, diuretic drugs and drugs for central

Table 2. Neonatal therapeutic interventions.

	Group II (n = 55)	Group III (n = 95)	
		Group IIIA (n = 50)	Group IIIB (n = 45)
Mechanical ventilation	0 (0)	44 (88.0)[a]	44 (97.8)[b]
nCPAP	8 (14.5)	6 (12.0)	1 (2.2)[b]
Inotropic drugs	10 (18.2)	15 (30.0)	36 (80.0)[b,c]
Dobutamine (≤ 5 mkg/kg/min)	10 (18.2)	15 (30.0)[a]	36 (80.0)[b]
Dobutamine (> 5 mkg/kg/min)	0 (0)	0 (0)	8 (17.8)[b,c]
Dopamine (≤ 5 mkg/kg/min)	0 (0)	0 (0)	4 (8.9)[b,c]
Dopamine (> 5 mkg/kg/min)	0 (0)	0 (0)	2 (4.4)
Dexamethasone	0 (0)	0 (0)	1 (2.2)
Diuretic drugs (loop)	5 (9.1)	9 (18.0)	24 (53.3)[b,c]
Drugs for CNS	0 (0)	18 (36.0)[a]	38 (84.4)[b,c]
Antibiotics			
Ampicillin	49 (89.1)	50 (100.0)	45 (100.0)[b]
Cefotaxime	8 (14.5)	16 (32.0)[a]	16 (35.6)[b]
Gentamicin/Amikacin	46 (83.6)	50 (100.0)[a]	45 (100.0)[b]
Plasma	0 (0)	9 (18.0)[a]	9 (20.0)[b]
Erythrocyte	0 (0)	5 (10.0)[a]	6 (13.3)[b]

All entries are n (%).
[a]Significant difference between groups II and IIIA, $p < 0.05$; [b]significant difference between groups II and IIIB, $p < 0.05$; [c]significant difference between groups IIIA and IIIB, $p < 0.05$.
nCPAP: nasal continuous positive airway pressure; CNS: central nervous system.

nervous system (CNS) was determined. It should be noted that most critically ill newborns from group III received complex antibiotic therapy including potential nephrotoxic aminoglycosides, and needed mechanical ventilation. The average value of nTISS during treatment in NICU in group II was 5.0 ± 1.48 points, in group IIIA – 12.3 ± 4.12 points, in group IIIB – 18.8 ± 4.51 points ($p_{II-IIIA} < 0.05$, $p_{II-IIIB} < 0.05$, $p_{IIIA-IIIB} < 0.05$).

Multiple clinical and laboratory signs of renal dysfunction were found in most neonates with disorders of early neonatal period (**Tab. 3**). In healthy newborns (group I), physiological leukocyturia was found in 11 (20.0%), flat epithelium in urea in 28 (50.9%), oxalates in urea in 35 (63.6%) neonates as signs of "transitional states" in early neonatal period without edema and pathological changes of body mass. The development of moderate perinatal disorders in full-term newborns was associated with pathological increase of body mass in 12 (21.8%) patients. As compared to group I, patients in group II were more often diagnosed with pathological changes in urine. These included pathological leukocyturia in 8 (14.5%) neonates, renal epithelium in 5 (9.1%) and granular cylinders in 3 (5.5%) neonates. 5 (10.0%) critically ill full-term newborns in group IIIA had edema and 9 (18.0%) newborns had pathological increase of body mass ($p_{IIIA-II} < 0.05$). Among 50 patients in this group, 3 (6.0%) newborns had pathological hematuria, 10 (20.0%) had pathological leukocyturia ($p_{IIIA-I} < 0.05$), 4 (8.0%) had renal epithelium, and 2 (4.0%) had granular cylinders. Certain association between AKI with edema and pathological increase of the body mass as well as with pathological hematuria (22.2%, $p_{IIIB-IIIA} < 0.05$), pathological leukocyturia (42.2%, $p_{IIIB-IIIA} < 0.05$), renal epithelium (31.1%, $p_{IIIB-IIIA} < 0.05$), and granular cylinders (13.3%, $p_{IIIB-IIIA} < 0.05$) was determined.

The results of measurement of biochemical serum and urine markers are presented in **Tab. 4**. The established marker of renal dysfunction SCr was significantly higher in the groups of critically ill newborns as compared to the group of patients with signs of moderate neonatal disorders. The AKI group displayed significantly higher values of SCr as compared to the non-AKI group. Accordingly, minimum mean values of GFR were found in critically ill newborns with AKI.

In addition, SUr was much higher in the group of severe neonatal disorders (group IIIA) as compared to healthy babies (group I) and patients with moderate neonatal disorders (group II). The maximum level of SUr was significantly higher in newborns with AKI (group IIIB).

Further data of renal excretion demonstrated that all full-term newborns with both stages of disorders of early neonatal period (groups II and III) had significantly lower level of UCr as compared to healthy neonates (group I). The healthy newborns had a high level of UUr as signs of "transitional states" in their first week of life. The highest level of UUr in both groups of patients with neonatal disorders was found in patients with

Table 3. Neonatal renal data.

	Group I (n = 55)	Group II (n = 55)	Group III (n = 95)	
			Group IIIA (n = 50)	Group IIIB (n = 45)
Edema	0 (0)	0 (0)	5 (10.0)[a,d]	15 (33.3)[b,c,d]
Pathological increase body weight (> 5%)	0 (0)	12 (21.8)[d]	9 (18.0)[d]	21 (46.7)[b,c,d]
Hematuria (0-2 erythrocytes)	0 (0)	6 (10.9)[d]	8 (16.0)[d]	8 (17.8)[b,d]
Hematuria (> 2 erythrocytes)	0 (0)	0 (0)	3 (6.0)	10 (22.2)[b,c,d]
Leukocyturia (0-5 leukocytes)	11 (20.0)	28 (50.9)[d]	15 (30.0)	8 (17.8)[b]
Leukocyturia (> 5 leukocytes)	0 (0)	8 (14.5)[d]	10 (20.0)[d]	19 (42.2)[b,d]
Flat epithelium	28 (50.9)	34 (61.8)	16 (32.0)	15 (33.3)[b]
Transitional epithelium	3 (5.5)	5 (9.1)[d]	8 (16.0)[d]	8 (17.8)[d]
Renal epithelium	0 (0)	5 (9.1)[d]	4 (8.0)[d]	19 (31.1)[b,c,d]
Hyaline cylinders	0 (0)	0 (0)	4 (8.0)[a,d]	6 (13.3)[b,d]
Granular cylinders	0 (0)	3 (5.5)	2 (4.0)	6 (13.3)[b,c,d]
Oxalates	35 (63.6)	20 (36.4)[d]	19 (38.0)	24 (53.3)

All entries are n (%).
[a]Significant difference between groups II and IIIA, $p < 0.05$; [b]significant difference between groups II and IIIB, $p < 0.05$; [c]significant difference between groups IIIA and IIIB, $p < 0.05$; [d]significant difference from group I, $p < 0.05$.

Table 4. Neonatal biochemical data.

	Group I (n = 55)	Group II (n = 55)	Group III (n = 95)	
			Group IIIA (n = 50)	Group IIIB (n = 45)
SCr, μmol/l, M ± SD (95% CI)	41.6 ± 8.49 (39.3-43.9)	45.7 ± 12.85 (42.2-49.2)	54.1 ± 11.78 [a,d] (50.7-57.4)	68.7 ± 15.56 [b,c,d] (64.1-73.4)
GFR, ml/min/1.73 m², M ± SD (95% CI)	53.7 ± 11.16 (50.6-56.7)	43.7 ± 8.78 [d] (41.3-46.1)	40.5 ± 8.14 [d] (38.2-42.8)	30.7 ± 8.33 [b,c,d] (28.1-33.2)
SUr, mmol/l, M ± SD (95% CI)	2.78 ± 0.61 (2.62-2.95)	2.82 ± 0.62 (2.65-2.98)	3.76 ± 1.18 [a,d] (3.28-4.29)	6.76 ± 2.18 [b,c,d] (5.69-7.56)
UCr, μmol/l, M ± SD (95% CI)	3,459.7 ± 1,167.26 (3,144.2-3,775.3)	1,676.6 ± 502.89 [d] (1,540.6-1,812.5)	2,006.8 ± 735.28 [a,d] (1,797.8-2,215.7)	1,311.6 ± 445.81 [c,d] (1,177.6-1,445.5)
UUr, mmol/l, M ± SD (95% CI)	48.4 ± 19.46 (43.1-53.6)	28.2 ± 10.47 [d] (25.4-31.1)	34.9 ± 5.6 [a,d] (33.3-36.5)	42.9 ± 9.25 [b,c] (40.1-45.6)
UTPr, mg/l, M ± SD (95% CI)	165.4 ± 64.75 (147.8-182.9)	127.3 ± 47.4 [d] (114.5-140.2)	142.5 ± 35.09 [d] (132.4-152.7)	185.5 ± 62.15 [b,c] (166.8-204.1)
UAlb, mg/l, M ± SD (95% CI)	10.5 ± 3.27 (9.59-11.37)	16.6 ± 6.01 [d] (14.9-18.3)	17.6 ± 5.38 [d] (16.1-19.1)	21.3 ± 8.63 [b,c,d] (18.8-23.7)
UIgG, mg/l, M ± SD (95% CI)	4.3 ± 1.19 (3.96-4.6)	4.75 ± 1.51 (4.34-5.15)	4.38 ± 0.74 (4.17-4.59)	5.73 ± 1.52 [b,c,d] (5.27-6.11)
Uα1-MG, mg/l, M ± SD (95% CI)	24.2 ± 9.87 (21.5-26.9)	30.5 ± 10.87 [d] (27.6-33.5)	32.8 ± 8.41 [d] (30.4-35.2)	42.7 ± 14.74 [b,c,d] (38.2-47.1)
Uβ2-MG, mg/l, M ± SD (95% CI)	2.38 ± 1.11 (2.08-2.68)	1.68 ± 0.63 [d] (1.52-1.86)	2.66 ± 0.94 [a] (2.4-2.93)	2.41 ± 1.07 [b] (2.08-2.73)

[a]Significant difference between groups II and IIIA, $p < 0.05$; [b]significant difference between groups II and IIIB, $p < 0.05$; [c]significant difference between groups IIIA and IIIB, $p < 0.05$; [d]significant difference from group I, $p < 0.05$.
SCr: serum creatinine; GFR: glomerular filtration rate; SUr: serum urea; UCr: urinary creatinine; UUr: urinary urea; UTPr: urinary total protein; UAlb: urinary albumine; UIgG: urinary immunoglobulin G; Uα1-MG: urinary α1-microglobulin; Uβ2-MG: urinary β2-microglobulin.

AKI. Critically ill newborns with AKI had lower level of UCr than any other groups.

As compared to healthy newborns, the patients with clinical signs of moderate neonatal disorders and severe disorders without AKI had significantly lower levels of mean UTPr (165.4 mg/l, 127.3 mg/l and 142.5 mg/l respectively, $p_{II\text{-}I} < 0.05$, $p_{IIIA\text{-}I} < 0.05$). The highest mean level of this urinary marker was found in critically ill newborns with AKI (185.5 mg/l, $p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$).

Sick neonates with neonatal disorders in groups II and IIIA had significantly higher levels of UAlb, a protein marker, than healthy newborns (16.6 mg/l, 17.6 mg/l and 10.5 mg/l respectively, $p_{II\text{-}I} < 0.05$, $p_{IIIA\text{-}I} < 0.05$). The highest levels of UAlb were found in patients with AKI (21.3 mg/l, $p_{IIIB\text{-}I} < 0.05$, $p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$).

No difference in UIgG was found between healthy newborns and neonates of groups II and IIIA (4.3 mg/l, 4.75 mg/l and 4.38 mg/l respectively, $p > 0.05$). Critically ill patients with AKI had significantly higher levels of this marker as compared to all other groups (5.73 mg/l, $p_{IIIB\text{-}I} < 0.05$, $p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} < 0.05$).

At the same time, the results of the study demonstrated associations between the level of Uα1-MG and degrees of neonatal disorders. As compared to healthy neonates, neonates with neonatal problems of moderate and severe degrees without AKI had higher α1-MG in urine (24.2 mg/l, 30.5 mg/l and 32.8 mg/l respectively, $p_{II\text{-}I} < 0.05$, $p_{IIIA\text{-}I} < 0.05$). The maximum of this index was found in newborns with AKI (42.7 mg/l, $p_{IIIB\text{-}I} < 0.05$, $p_{IIIB\text{-}II} < 0.05$ $p_{IIIB\text{-}IIIA} < 0.05$).

Neonates who had clinical signs of moderate disorders of early neonatal period had significantly lower level of Uβ2-MG than healthy neonates (1.68 mg/l vs 2.38 mg/l, $p_{II\text{-}I} < 0.05$). Critically ill newborns with severe neonatal disorders had significantly higher level of this urinary biomarker but without significant difference between subgroups (2.66 mg/l in group IIIA and 2.41 mg/l in group IIIB, $p_{IIIA\text{-}II} < 0.05$, $p_{IIIB\text{-}II} < 0.05$, $p_{IIIB\text{-}IIIA} > 0.05$).

Discussion

Proteins are found excreted in urine as a result of their glomerular filtration and reabsorption in

the proximal tubules. The concentration of proteins filtered in renal glomeruli depends on several factors, including renal plasma flow, protein concentration in the afferent part of glomerular vessels, hydrostatic and oncotic pressure on both sides of the filtration membrane, size, shape and static charge of protein, as well as the properties of the filtration barrier [11, 12]. Protein reabsorption occurs through receptor-mediated endocytosis, mainly in the convoluted part and the initial segment of the straight part of proximal tubules. Many proteins bound to cell membranes (in the apical part of cells) were found to be a part of the mechanism of reabsorption. However, not all products of protein hydrolysis entering renal tubule cells are reintroduced into circulation. Moreover, maturation of the filtration barrier is faster than maturation of the tubular cells [13].

Urinary biomarkers of renal dysfunction in newborns have been investigated extensively, and in this study several protein markers were detected for evaluation at both levels: glomerular filtration and tubular reabsorption.

The mean value of UTPr in groups of patients with moderate disorders in the first week of life and critically ill newborns without AKI was found to be significantly lower as compared to healthy newborns. This fact may be due to the presence of "transient proteinuria" in healthy newborns during the first 5 days of life. The main causes of this transient status are increasing permeability of the glomerular and tubular epithelium, and capillaries [14].

In our study the highest level of UTPr was detected in patients with severe neonatal disorders and AKI. These findings accord with Westhoff et al., who demonstrated a high level of urinary protein in a paediatric group with AKI as compared to sick babies without AKI [15].

However, in our study the urinary concentration of UTPr was measured by means of protein dye-binding method. Although heterogeneous types of proteins and different molecular forms of proteins (such as albumin) may be present in urine, each of them has a specific affinity for the dye-binding process. It makes for a challenge to both accuracy and precision of measurement, and it may not accurately measure all clinically significant types of proteinuria. Both pre-analytical factors and factors intrinsic to the analysis itself can be sources of error in UTPr measurement. Currently there is no reference measurement procedure or standardized reference material for urinary protein [16]. Added to literature data, results of this study demonstrated that dye-binding method for measuring UTPr does not have high level of reliability and cannot be recommended for practical use.

Many guidelines recommend the measurement of UAlb on the basis of the need to detect lower levels of protein than were previously thought to be clinically significant [17, 18]. Measures of UTPr are imprecise at low levels of protein and are insensitive at detecting clinically important changes in albuminuria. Relatively large increases in UAlb excretion can occur without causing a measurable increase in UTPr. UAlb measurements are more specific and more sensitive for changes in glomerular permeability than those of UTPr. Additionally, since a single protein is measured, standardization of albumin measurement is simpler than standardization of UTPr measurements [16].

Our results demonstrated that not only newborns with AKI but also patients in neonatal intensive care with signs of moderate neonatal disorders and critically ill neonates without AKI had higher levels of UAlb than healthy newborns.

Albumin loss in urine has long been used as a marker of kidney injury, whether it originates from glomerular dysfunction, defective total protein reabsorption, or their combination [19]. Elevated levels of UAlb were found in preterm neonates with asphyxia and respiratory distress and it was suggested as a possible biomarker of AKI in newborns [7] and preterm neonates [20]. Sellmer et al. found that UAlb is associated with patent ductus arteriosus (PDA) and its size, even after adjusting for gestational age, gender, and sepsis [21].

Our study presents the evidence that critically ill newborns with AKI had significantly higher level of IgG as compared to other groups. It does not penetrate an intact glomerular membrane, and is not detected in the urine of healthy individuals [10, 11].

High concentration of Alb and IgG in the urine of newborns with signs of AKI was found to imply the development of non-selective proteinuria with a loss of glomerular barrier properties. The morphological basis of functional disorders of glomerular filter is damaged on thin legs of podocytes, which are intertwined with each other, coalesce and form a continuous layer of cytoplasm covering the basement membrane of the glomeruli. The other main pathological mechanisms are damage of structural components of the glomerular membrane by proteolytic enzymes, reactive oxy-

gen species, inflammatory cells and proteins directly in the wall filter [11, 13].

In addition, certain association between the level of α1-MG and the stage of neonatal disorders and renal dysfunction was found. In comparison with healthy newborns, patients with moderate disorders and critically ill neonates without and with AKI had significantly higher levels of α1-MG. The highest level of this marker was determined in patients with AKI.

According to Adiyanti and Loho, α1-MG is a sensitive biomarker for proximal tubular dysfunction, even for its early stage when no histological damage can be observed [22]. This is in accord with other researchers who claim that Uα1-MG might be a valuable indicator for evaluating early renal dysfunction in neonatal patients with HIE [23] or after neonatal asphyxia [24]. In comparison with β2-MG, α1-MG is more stable over a range of pH levels in the urine, making it a more useful urinary biomarker [16].

A significant difference of Uβ2-MG between full-term newborns without and with AKI was not found in the study. Although, significantly high level of this biomarker in the common group of critically ill patients was determined, as compared to newborns with moderate disorders in their first week of life.

β2-MG is filtered freely through the glomeruli of the kidney because it is small. Most of β2-MG in the filtrate is reabsorbed and catabolized by renal proximal tubular cells. Only trace amounts of β2-MG remain in urine and are excreted. Elevated level of serum β2-MG is indicative of glomerular malfunction, while elevated Uβ2-MG suggests tubular dysfunction [25, 26].

Initially, the increase in β2-MG levels in case of kidney injury was considered to be solely due to the decline in kidney function, but later studies have shown that other factors, including increased synthesis of β2-MG, may contribute to the change of result in these patients. Another significant drawback associated with the use of Uβ2-MG as a marker of kidney injury is its instability in urine at room temperature, particularly when pH is less than 5.5; for this reason, urine should be alkalinized and frozen at -80°C immediately after collection [16].

Measurement of Uβ2-MG has been proposed as a convenient and sensitive approach for screening patients with kidney injury, primarily for patients with tubular injury but also for those with glomerular disease [26, 27]. Saeidi et al. have recently described an association of UAlb with sex, and Uβ2-MG with postnatal age in a group of premature neonates [28]. Chaudhary et al. demonstrated that β2-MG is a sensitive indicator of renal disease and can detect subclinical renal involvement in meconium aspiration syndrome [25]. Banerjee et al. showed that urinary β2-MG level may be related to HIE in full-term patients [29]. Askenazi et al. tested 12 biomarkers in infants with very low birth weight with and without AKI. They found that UAlb and Uβ2-MG were not statistically significantly related when looking only at the biomarkers, but became statistically significantly different after controlling for UCr [7].

In addition, the results of our study demonstrated absolute reliability of Uα1-MG for identification of AKI in neonatal cohort, as opposed to Uβ2-MG, considering that this marker is not influenced by extra-renal factors and is stable in urine with acid pH. It should be noted that laboratory methods for measuring these proteins are robust. Therefore, authors recommend measuring of Uα1-MG as a marker of renal tubular injury in newborns.

Our study has several restrictions. First, it is a single-center study in a relatively small patient cohort. A larger cohort is needed to relate the stage of neonatal disorders and modern early markers of renal dysfunction in sick newborns. Second, it would be useful to indicate urinary protein markers between different types of AKI (pre-renal, renal and post-renal). Third, the diagnostic value of these markers should be studied and diagnosis models created for indication of renal dysfunction in sick newborns, not only in those with AKI. This is our objective for the future.

Conclusion

The study showed that manifestations of renal disease in full-term newborns are related to adverse perinatal conditions. In comparison with healthy newborns, neonates with moderate disorders in the early neonatal period had dysfunction of the glomerular membrane (selective proteinuria with excessive excretion of UAlb) and proximal tubules (increased Uα1-MG). More severe neonatal disorders in term newborns were accompanied also by signs of the presence of selective proteinuria along with more significant tubular failure (excessive excretion of α1-MG and β2-MG). Formation of AKI in term infants is associated with complex disorders of all structural elements of the nephron, which is manifested by extremely high levels of total protein markers of glomerular

(UTPr, UAlb and UIgG) and tubular (α1-MG and β2-MG) dysfunction in urine. It should be noted that detection of UAlb and α1-MG levels is the most informative diagnostic marker of AKI in critically sick neonates.

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Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Chaturvedi S, Ng KH, Mammen C. The path to chronic disease following acute kidney injury: a neonatal perspective. Pediatr Nephrol. 2017;32(2):227-41.
2. Mussap M, Noto A, Fanos V, Van Den Anker JN. Emerging biomarkers and metabolomics for assessing toxic nephropathy and acute kidney injury (AKI) in neonatology. Biomed Res Int. 2014;2014:602526.
3. Ottonello G, Dessì A, Neroni P, Trudu ME, Manus D, Fanos V. Acute kidney injury in neonatal age. J Pediatr Neonat Individual Med. 2014;3(2):e030246.
4. Askenazi DJ, Koralkar R, Hundley HE, Montesanti A, Parwar P, Sonjara S, Ambalavanan N. Urine biomarkers predict acute kidney injury in newborns. J Pediatr. 2012;161(2):270-5.
5. Gopal G. Acute Kidney Injury (AKI) in perinatal asphyxia. Indian J Pharm Biol Res. 2014;2(2):60-5.
6. Selewski DT, Charlton JR, Jetton JG, Guillet R, Mhanna MJ, Askenazi DJ, Kent AL. Neonatal acute kidney injury. Pediatrics. 2015;136(3):e463-73.
7. Askenazi DJ, Koralkar R, Patil N, Halloran B, Ambalavanan N, Griffin R. Acute kidney injury urine biomarkers in very low-birth-weight infants. Clin J Am Soc Nephrol. 2016;11(9): 1527-35.
8. Endre ZH, Kellum JA, Di Somma S, Doi K, Goldstein SL, Koyner JL, Macedo E, Mehta RL, Murray PT. Differential diagnosis of AKI in clinical practice by functional and damage biomarkers: workgroup statements from the tenth Acute Dialysis Quality Initiative Consensus Conference. Contrib Nephrol. 2013;182:30-44.
9. Richardson DK, Gray JE, McCormick MC, Workman K, Goldmann DA. Score for Neonatal Acute Physiology: a physiologic severity index for neonatal intensive care. Pediatrics. 1993;91(3):617-23.
10. Bagdasarova IV, Fomina SP. [Chronic kidney disease in children and renal replacement therapy in Ukraine]. [Article in Ukrainian]. Ukr J Nephrol Dial. 2015;1(45):3-7.
11. Shayman JA. Renal pathophysiology. New York: Lippincott-Raven, 2010.
12. De Loor J, Daminet S, Smets P, Maddens B, Meyer E. Urinary biomarkers for acute kidney injury in dogs. J Vet Intern Med. 2013;27(5):998-1010.
13. Peres GB, Michelacci YM. The role of proximal tubular cells in the early stages of diabetic nephropathy. J Diabetes Metab. 2015;6:551.
14. Blackburn ST. Renal function in the neonate. J Perinat Neonatal Nurs. 1994;8(1):37-47.
15. Westhoff JH, Tönshoff B, Waldherr S, Pöschl J, Teufel U, Westhoff TH, Fichtner A. Urinary tissue inhibitor of Metalloproteinase-2 (TIMP-2) • Insulin-Like Growth Factor-Binding Protein 7 (IGFBP7) predicts adverse outcome in pediatric acute kidney injury. PLoS ONE. 2015;10(11): e0143628.
16. Skorecki K, Chertow GM, Marsden PA, Taal MW, Yu ASL. Brenner & Rector's The Kidney. Philadelphia, PA: Elsevier, 2016.
17. Johnson DW, Jones GR, Mathew TH, Ludlow MJ, Chadban SJ, Usherwood T, Polkinghorne K, Colgiuri S, Jerums G, MacIsaac R, Martin H; Australasian Proteinuria Consensus Working Group. Chronic kidney disease and measurement of albuminuria or proteinuria: a position statement. Med J Aust. 2012;197(4):224-5.
18. KDIGO. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2013;3(1):1-150.
19. Dickson LE, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really! J Am Soc Nephrol. 2014;25(3):443-53.
20. DeFreitas MJ, Seeherunvong W, Katsoufis CP, RamachandraRao S, Duara S, Yasin S, Zilleruelo G, Rodriguez MM, Abitbol CL. Longitudinal patterns of urine biomarkers in infants across gestational ages. Pediatr Nephrol. 2016;31(7):1179-88.
21. Sellmer A, Bech BH, Bjerre JB, Schmidt MR, Hjortdal VE, Esberg G, Ritting S, Henriksen TB Urinary neutrophil gelatinase-associated lipocalin in the evaluation of patent ductus arteriosus and AKI in very preterm neonates: a cohort study. BMC Pediatr. 2017;17(1):7.
22. Adiyanti SS, Loho T. Acute kidney injury (AKI) biomarker. Acta Med Indones. 2012;44(3):246-55.
23. Li L. [Significance of urinary α1-MG in renal injury of HIE]. [Article in Chinese]. Chin J Trauma Disability Med. 2009;5:31-3.
24. Dong C-X, Zhang L-M, Zhang A-M. [The significance of urine α1-microglobulin in renal injury after neonatal asphyxia]. [Article in Chinese]. Chin J Clin Rational Drug Use. 2010;4: 50-1.
25. Chaudhary GS, Chaudhary V, Dagar S. Evaluation of renal dysfunction by Beta-2 Microglobulinuria in neonates with meconium aspiration syndrome. J Adv Res Biol Sci. 2011;3(2):16-9.

26. Zeng X, Hossain D, Bostwick DG, Herrera GA, Ballester B, Zhang PL. Urinary β2-Microglobulin is a sensitive indicator for renal tubular injury. SAJ Case Rep. 2014;1(1):103.
27. Aydin B, Dilli B, Erol S, Ozyazici E, Beken S, Cullas Ilarslan NE, Zenciroglu A, Okumus N. 1081 levels of serum N-Terminal Pro-Brain Natriuretic Peptide, Cystatin C, and Urinary B2 Microglobulin in newborns with hypoxic ischemic encephalopathy. Arch Dis Child. 2012;97(Suppl 2):A310.
28. Saeidi B, Koralkar R, Griffin RL, Halloran B, Ambalavanan N, Askenazi DJ. Impact of gestational age, sex, and postnatal age on urine biomarkers in premature neonates. Pediatr Nephrol. 2015;30(11):2037-44.
29. Banerjee M, Majumdar SK, Shahidullah MD. Relationships of urinary β2-microglobulin in neonates with impaired renal function in different stages of hypoxic ischaemic encephalopathy. Bangladesh J Child Health. 2013;37(1):22-6.