ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Assessment of the diagnostic ability of RIFLE classification and neutrophil gelatinase-associated lipocalin biomarker in detecting acute kidney injury in newborns in the intensive care unit

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SUMMARY

Introduction/Objective This study was designed to demonstrate the association of the RIFLE classification and neutrophil gelatinase-associated lipocalin (NGAL) in predicting of newborns with acute kidney injury (AKI).

Methods This was a prospective study. We included 100 newborns suspected of having a kidney injury. These newborns were admitted to the intensive care unit (ICU) at the University Clinic of Pediatrics from the period of two years. The severity of the disease was determined by RIFLE classification. The biochemical marker NGAL was included in this study because it is an early biomarker of AKI in newborns. The statistical processing of the material was by methods of descriptive statistics.

Results The prevalence rate of AKI was 6.25%, but according to the RIFLE classification the prevalence was 8.7%. According to RIFLE classification, we reported "risk" in 36%, "injury" in 50% and "failure" in 14% of newborns with AKI. In newborns with perinatal asphyxia, kidney injury was seen in 34% and 30%, making perinatal asphyxia the most common predisposing factor. The difference in average value of the score for neonatal acute physiology with perinatal extension in newborns with AKI and the control group without AKI was confirmed significant (p < 0.001). Also, there was a significant difference (p < 0.001) between serum creatinine and urinary NGAL values, on the day they were admitted to the ICU.

Conclusion In newborns hospitalized in the ICU, AKI is a serious condition. We could identify kidney injury and follow up the progression of the disease by using RIFLE classification. The need for early diagnosis of kidney injury, in a period when the disease is not clinically manifest, in the first hours of its occurrence, is provided by NGAL.

Keywords: acute kidney injury; newborns; RIFLE classification; NGAL

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INTRODUCTION

Acute kidney injury (AKI) involves a sudden impairment of kidney function, leading to an imbalance of electrolytes, fluids, and waste products [1]. This complex disorder of kidney function can present with a variety of clinical manifestations. It can manifest as a kidney injury requiring replacement therapy or as minimal kidney dysfunction. In newborns who are critically ill, AKI is a common clinical condition. The reason for this is the immaturity of the kidneys in newborns, making them sensitive to reduced kidney function [2, 3].

Due to the need of timely and appropriate diagnosis as well as assessment of the severity of kidney injury, Risk Injury Failure Loss End-stage renal disease (RIFLE) classification is used in newborns with AKI. RIFLE classification covers the following stages: risk of kidney failure, kidney injury, kidney failure, kidney function loss and end-stage kidney failure). Taking into account the immaturity of tubular cells, high body water content and presence of maternal creatinine in newborn's circulation,

Bezzero et al. [4] have modified RIFLE classification to a newborns RIFLE classification. In this classification, the corrected values of serum creatinine and 24-hour urinary output are taken as criteria [4].

AKI occurs in 8–24% of newborns. The mortality rate is 10–61% [5]. Clinical conditions in newborns such as perinatal asphyxia, prematurity, congenital heart disease, sepsis, and meconium plug syndrome are factors that predispose to AKI. The risk of kidney injury in newborns hospitalized at the intensive care unit (ICU) is reduced with appropriate and timely treatment of these associated comorbidities [6, 7].

The scoring system most commonly used in newborns as a tool for predicting severity of illnesses is Score for Neonatal Acute Physiology with Perinatal Extension 2 (SNAPPE 2) score. This score needs to be performed within the first 24 hours of the newborn's admission to the ICU. The SNAPPE 2 score system is used to assess the severity of the disease and is correlated with newborns mortality in ICUs. A score above 40 is associated with a higher mortality rate [8].

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In newborns, serum creatinine as a parameter for glomerular filtration rate is not used. The reason for this is that in newborns serum creatinine shows higher values in the first 2–3 days of birth, because those values reflect the function of the mother's kidneys. Serum creatinine values in the following days they subsequently decrease [9]. Serum creatinine levels also change 48–72 hours after the onset of kidney injury. So, the serum value of creatinine is considered a late marker for kidney injury [10, 11]. Neutrophil gelatinase-associated lipocalin (NGAL) is used as a biomarker to detect kidney injury in the first two to three hours after onset. Kidney damage can be detected with the help of NGAL even before there is a decrease in urine output and before an increase in serum creatinine. That is why NGAL is the most appropriate biomarker for early detection of kidney injury in critically ill newborns.

The severity of the disease correlates with serum and urine NGAL values, and are dependent on the extent of current kidney injury. Its clinical use ensures that we make the right clinical decisions before the disease manifests itself [12, 13, 14]. The purpose of this study was to analysis the role of RIFLE classification and (NGAL) in predicting AKI in newborns.

METHODS

Study population

The study was a prospective and we included 100 newborns suspected for kidney injury admitted at the ICU, at the University Clinic of Pediatrics from the period of two years. The newborns were divided into a group with AKI, included 50 newborns, and the control group included 50 newborns without AKI. Inclusion criteria in this study were newborns up to 28 days after birth, who were admitted at the ICU due to certain pathological condition, with or without the development of AKI. In newborns less than 33 weeks of gestation, an increase in serum creatinine level above 130 μmol/l, as well as a serum creatinine value greater than 90 μmol/l in newborns older than 33 weeks of gestation was defined as AKI. Oliguria was defined as a urinary output less than 1.0 ml/kg/h. All newborns who were less than 25 weeks of gestation according to our criteria were excluded from the study.

According to the criteria for classification Neonatal RIFLE, gestational age, birth weight, gender and predisposing factors such as asphyxia, prematurity, sepsis, congenital heart diseases and meconium plague syndrome, were analyzed medical data from hospitalized newborns. With SNAPPE 2 achieved in the first 12 hours after admission at the ICU, the severity of the disease of the admitted newborns was assessed.

For all studied patients we have the approval of the institutional committee on ethics.

Study design

In the neonates included in the study, the severity of the disease was determined based on the neonatal RIFLE classification using the criteria shown in Table 1.

Table 1. Neonatal RIFLE classification criteria

Stage	Criteria	Urine output
R (risk)	> 1.5 Cr, GFR < 25%	< 1.0 ml/kg/24 h, term neonates < 1.5 ml/kg/24 h, in premature
I (injury)	> 2 Cr, GFR < 50%	< 1.0 ml/kg/24 h
F (failure)	> 3 Cr, GFR < 75%	< 0.7 ml/kg/24 h or anuria 12 h
L(loss)	AKI > 1 month	
E (end stage)	AKI > 3 month	

GFR – glomerular filtration rate; AKI – acute kidney injury

The laboratory tests were done at the Clinical Laboratory at the University Clinic of Pediatrics and in the Institute of biochemistry Faculty of Medicine, Skopje. Laboratory test which included serum creatinine, and urinary NGAL were taken first at the admission, and after 72 hours. The collected urine samples were frozen at -70 to -80°C, before being transferred to the Institute of Biochemistry.

Using the biochemical analyzer Architect c4000 Abbott (Mmol/L) and the NGAL biomarker, using the NGAL ELISA method (Bioporto, Hellerup, Denmark) ng/L, the laboratory values of serum creatinine were examined.

Statistical analysis

The statistical processing of the material was by the methods of descriptive statistics. Independent sample tests were used to determine the significance of the difference in the samples. Statistical significance was established for the values of $p < 0.05$.

RESULTS

During the study, we have evaluated 100 newborns hospitalized at the University Clinic of Pediatrics, during the two-year-long period. The AKI group included 50 newborns, and without AKI group, included 50 newborns with various pathological conditions.

In the AKI group 62% were male and 38% female. Comparable values with the control group of newborns, without AKI group, were 56% males and 44% females, retrospectively. Most of involved newborns in AKI and without AKI group were born at term (61% and 57%). In newborns with AKI, the average gestational age was 36.152 ± 4.2 weeks of gestation and 35.26 ± 2.7 weeks of gestation in control group. In newborns with AKI, the average birth weight was 2660.5 ± 458.1 grams, while in the control group was 2489.4 ± 564.8 grams. The average serum creatinine values in AKI group was 184.44 ± 103.74 μmol/l, and 77.78 ± 30.6 μmol/l in control group. The mean urine output values in AKI group was

Table 2. Demographic characteristics of newborns with acute kidney injury and non-acute kidney injury

Parameters	Acute kidney injury	Non acute kidney injury
Male	31 (62%)	28 (56%)
Female	19 (38%)	22 (44%)
Mean ± SD gestation age (week)	36.15 ± 4.2	35.26 ± 2.7
Mean ± SD birth weight	2660.5 ± 458.1	2489.4 ± 564.8
Duration of stay (days)	17.82 ± 8.4	13.38 ± 7.7
Mean ± SD serum creatinine	184.44 ± 103.74 µmol/l	76.78 ± 30.6 µmol/l
Mean ± SD urine output	1.14 ± 0.8 ml/kg/h	3.49 ± 1.5 ml/kg/h

Table 4. Predisposing factors in newborns with acute kidney injury and control group without acute kidney injury

Predisposing factors	Acute kidney injury N / %	Without acute kidney injury N / %
Asphyxia	17 (34%)	15 (30%)
Sepsis	13 (26%)	12 (24%)
Preterm	12 (24%)	14 (28%)
Congenital heart disease	3 (6%)	5 (10%)
Meconium plug syndrome	3 (6%)	5 (10%)

Table 5. Distribution of newborns with acute kidney injury and control group according to the RIFLE classification

RIFLE classification	Acute kidney injury N / %	without acute kidney injury N / %
Risk	18 (36%)	15 (30%)
Injury	25 (50%)	3 (6%)
Failure	7 (14%)	0

1.14 ± 0.8 ml/kg/h and 3.49 ± 1.5 ml/kg/h in control group. Demographic characteristics of newborns with AKI and non AKI are summarized in Table 2.

In the first 12 hours of ICU hospitalization, all newborns were analyzed with the SNAPPE 2 score. The results of the score were evaluated in four categories: very severe (over 70), severe (41–70), medium (score 21–40) and light (score 1–20).

All newborns were analyzed by SNAPPE 2 score within the first 12 hours of hospitalization in NICU. The score values were evaluated in four categories: light (score 1–20), medium (score 21–40), severe (41–70) and very severe (over 70). The results showed that the value of the average score of newborns with AKI was 54.72 ± 35.3, while 38 ± 10 in control group.

The difference between the AKI group and the control group was 16.72 and it was confirmed as significant p < 0.001.

The distribution of newborns with AKI depending of score levels compared to control group without AKI. In 50% newborns with AKI predominate severe score level, while in control group predominate median score level in 42% of newborns show in Table 3.

Table 3. Distribution of newborns with acute kidney injury (AKI) depending of score levels compared to control group

Score	AKI / without AKI	Mean ± SD	Min–Max
Light	AKI	0	0
	without AKI	13.7 ± 3.2	9–19
Medium	AKI	31.5 ± 4.4	28–39
	without AKI	27.6 ± 5.7	21–38
Severe	AKI	54.41 ± 7	43–69
	without AKI	41.21 ± 5.8	36–51
Very severe	AKI	89.45 ± 8.3	77–95
	without AKI	80.46 ± 8.3	70–93

Distribution of predisposing factors among newborns with AKI and control group are shown in Table 4. The most common contributing condition reported in both groups (34% *vs.* 30%) was perinatal asphyxia.

The RIFLE classification, which categorizes the severity of kidney injury, was implemented in the examined AKI group and control group without AKI. We founded

"risk" in 36% (18/50) of newborns with AKI, "injury" in 50% (25/50) and "failure" in 14% (7/50) of newborns. Additionally, using this classification, kidney injury was detected in the non AKI group too, with 30% (15/50) registering "risk" and 6% (3/50) "failure." Distribution of newborns with AKI compared to non AKI group in accordance with the RIFLE classification show in Table 5.

In 18 newborns with AKI, in whom we registered risk (R) in 78% (14/18), progression of disease occurred, with injury (I) registered in 62% (11/18) of newborns and failure (F) in 17% (3/18) of newborns. Only 19% (2/11) of the newborns with AKI in whom we reported subsequent injury (I), the experienced progressed of the disease to renal failure (F). From here, according to the standard definition of AKI in newborns the calculated prevalence was 6.25%, while according to the RIFLE classification it was 8.7%.
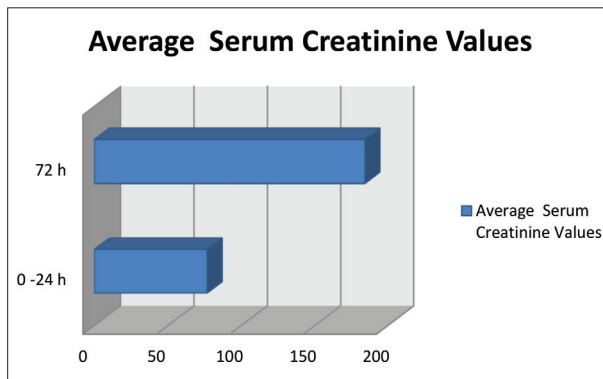
The results of the study, on the day of ICU admission showed that the serum creatinine level was normal in newborn with AKI and it was 76.78 ± 30.6 µmol/L and had an upward trend of 184.44 ± 103.74 µmol/L after 72 hours (Figure 1).

On the day of admission to the ICU in neonates with AKI the values of urinary NGAL the results in this study showed higher values (373.8 ± 194.9) and a slight upward trend, while on the third day after admission the results showed an additional increase (439.4 ± 254.7) p < 0.001 (Figure 2).

There was a significant difference p < 0.001 between serum creatinine and urinary NGAL values on the day of admission to the ICU.

DISSCUSION

The study presents a clinical, epidemiological investigation that evaluated in 100 newborns suspected for kidney injury admitted at the ICU at the University Clinic of Pediatrics, from the period of two years. The newborns were divided into examined and control group. The examined group included 50 newborns with AKI and the control group

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Figure 1. Serum creatinine value distribution in newborns with acute kidney injury overtime
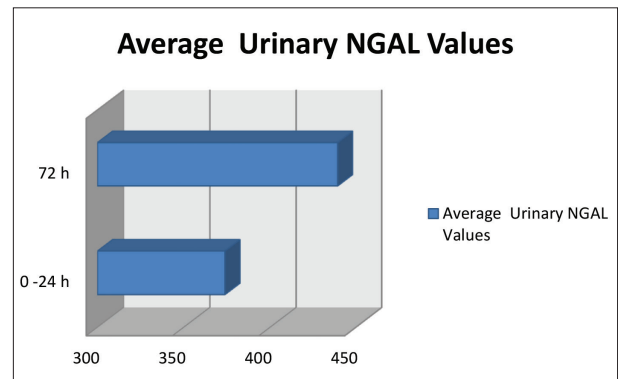
Figure 2. Urinary neutrophil gelatinase-associated lipocalin (NGAL) values distribution in newborns with acute kidney injury overtime

included 50 newborns without AKI with various pathological conditions [15].

According to the standard definition of AKI in newborns the calculated prevalence was 6.25%, which is consistent with literature data [16, 17]. A similar finding has been published from the study conducted by Vachvanichsanong et al. [18] where the prevalence of AKI in newborns was 6.3%, where in the study by Bolat et al. [19] it was 8%. There are opposite findings too. Mortazavi et al. [20] report on the prevalence of AKI of 2.7%, and Agras et al. [21] of 3.4%. We hypothesize that these differences may be due to differences in the diagnostic criteria for detecting kidney injury in newborns. According to the RIFLE classification, the prevalence of kidney injury in our study was 8.7%. This suggests that the diagnosis of AKI according to the standard classification may be missed in most newborns. Less of 36% of newborns with AKI had "risk," 50% "injury" and 14% had "renal failure." In 36% of newborns with registered risk, there was a progression of disease to injury in 62% and to "failure" in 17% of newborns. In 19% of newborns with verified "injury," the condition progressed to "renal failure." A similar finding is presented in the study by Mohkam et al. [22], in which, according to RIFLE criteria, 43% of newborns with AKI were at risk, 51% at injury and 6% at failure [23]. RIFLE classification was also applied in control group, newborns without AKI. The "risk" for AKI was present in 30% of newborns, while the "injury" in 6%. We did not record clinical progression of the condition and development of kidney injury in this group of newborns. This finding suggests that in control group, with verified "risk" and "injury," we could have overlooked the situation. We assume that the resolution of the kidney injury will come with the treatment of other indications. This suggests that the RIFLE classification in newborns can be used as a more sensitive method than the standard one in terms of diagnosis and monitoring of kidney injury [24]. There was a significant difference (p = 0.00001) between average SNAPP II score value in newborns with AKI compare to control group. In our study, newborns with AKI have had severe SNAPPE 2 score. The high score level was significantly associated with the severity of the disease. Critically ill newborns with AKI and other predisposing factors were significantly associated with high level score and poor prognosis. This finding correlates with the data presented in the study of Mortazavi et al. [20]. The poor prognosis was significantly higher in newborns with severe score in admission to the ICU, in whose further predisposing factors develop [25].

For all gestational ages according to gender distribution, in both groups of newborns most were male (62% and 56%). In terms of distribution by gestational age, most newborns were born term (64% and 54%). The most common predisposing factor in examined and control group perinatal asphyxia was observed in 34% and 30% of neonates, with a predominance of term male newborns, who were born with a low Apgar score in the fifth minute of life. Abu-Haweleh et al. [26] and Mortazavi et al. [20] published similar findings in their studies as well. Abu-Haweleh et al. [26] reports that 42% and Mortazavi et al. [20] reports that 30% of the newborns with AKI have had asphyxia as a dominant predisposing factor. In critically ill newborns on the day of ICU admission, in our study we found significantly higher urinary NGAL values compared to serum creatinine levels. This demonstrates that NGAL is a sensitive biomarker in the diagnosis of kidney injury. These data correlate with the data reported by Nickolas et al. [27] and Youssef et al. [28], emphasizing the role of NGAL as a sensitive marker in detecting kidney injury. Monitoring of urinary NGAL on the day of neonatal hospitalization of newborns in the ICU and 72 hours later showed an increasing trend in the average value. A high level of NGAL on initial measurement suggests that the kidney injury may have occurred prior to the newborns' hospitalization in the ICU. Namely, all critically sick newborns are transported to our unit, as a specialized tertiary center for intensive therapy. Intensive care explains the presence of renal injury in critically ill neonates in whom AKI is not yet clinically manifest. The role of urinary NGAL as an early biomarker for kidney injury is confirmed by its high values noted upon admission to the ICU. Mishra et al. [29] and Devarajan [30] in their studies also refer the ability of NGAL to early detect the newborns at risk of AKI.

CONCLUSION

AKI is a serious condition in hospitalized newborns in ICU. The severity of the disease can be assessed using the

SNAPPE 2 score. Kidney injury could be identified and disease progression monitored using the RIFLE classification. Early diagnosis of kidney injury in the first hours after its onset is made possible by NGAL, even when the disease is not clinically manifest.

Conflict of interest: None declared.

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Процена дијагностичке способности класификације РИФЛЕ и биомаркера липокалина везаног за неутрофилну гелатиназу у откривању акутне повреде бубрега код новорођенчади у јединици интензивне неге

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САЖЕТАК

Увод/Циљ Циљ студије био је да се испита улога класификације РИФЛЕ и липокалина везаног за неутрофилну гелатиназу у предвиђању акутне повреде бубрега (АПБ) код новорођенчади.

Методе У проспективној студији било је укључено 100 новорођенчади са суспектним бубрежним оштећењем примљених у јединицу интензивне неге на Универзитетској клиници за педијатрију у периоду од две године. Тежина АПБ утврђена је класификацијом РИФЛЕ. У студији је примењен липокалин везан за неутрофилну гелатиназу као рани биомаркер АПБ код новорођенчади. Материјал је статистички обрађен методама дескриптивне статистике.

Резултати Процењена преваленција АПБ била је 6,25%, док је преваленција према класификацији РИФЛЕ била 8,7%. Према класификацији РИФЛЕ, дијагностиковали смо „ризик" код 36%, „повреду" код 50% и „инсуфицијенцију" код 14% новорођенчади са АПБ. Најчешћи предиспонирајући фактор повезан са оштећењем бубрега била је перинатална асфик-

сија примећена код 34% и 30% новорођенчади. Потврђена је сигнификантна разлика између средње вредности скора за неонаталну акутну физиологију перинаталне екстензије код новорођенчади са АПБ и контролне групе без АПБ ($p < 0,001$). Постојала је значајна разлика ($p < 0,001$) између вредности креатинина у серуму и липокалина везаног за неутрофилну гелатиназу у урину на дан пријема у јединицу интензивне неге.

Закључак АПБ је озбиљно стање код хоспитализоване новорођенчади у јединици интензивне неге. Помоћу класификације РИФЛЕ могли бисмо идентификовати повреду бубрега и пратити напредовање болести. Биомаркер липокалин везан за неутрофилну гелатиназу пружа рану дијагнозу повреде бубрега у првим сатима настанка, када болест још увек није клинички јасна.

Кључне речи: акутна повреда бубрега; новорођенчад; класификација РИФЛЕ; липокалин везан за неутрофилну гелатиназу