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ACUTE KIDNEY INJURY IN NEONATES AND NEUTROPHILIC GELATINOUS ASSOCIATED LIPOCALIN AS EARLY BIOMARKER

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Abstract

The aim of the study was to determine the role of biomarker NGAL in early detection of kidney injury in neonates. The study evaluated the neonates suffering kidney injury who at the period of three years were treated at the University Children's Hospital in Skopje. All cases of neonates with kidney injury were analyzed according to gender, gestational age, birth weight and risk factors such as asphyxia, sepsis, prematurity, meconium plug syndrome and congenital heart diseases. NGAL was analyzed in urine samples collected on two occasions (day of admission and 2 days later) and the concentration of NGAL was determined using NGAL ELISA KIT (Bioporto). Medical data records of admitted neonates with AKI were analyzed. The material was statistically processed using methods of descriptive statistics. The study was carried out at the neonatal intensive care unit at the University Children's Hospital Skopje in which neonates with documented acute kidney injury were evaluated. The whole study has been performed during the 3 year period. The estimated prevalence of AKI in neonates was 6.4%. Most of the involved neonates in the study were born at term (68%) with predominance of male neonates (64%). The analyzed results showed a higher values of urinary NGAL on the day of admission (373.8 ± 194.9) and a slight upward trend, with further increase in the third day after admission (439.4 ± 254, 7). There was a significant difference between the uNGAL values and sCr values on the day of admission of neonates in NICU, $p<0,001$. The mean urinary NGAL values in neonates with AKI with lethal outcome were 586.39 ± 182.3 while the mean values in neonates without lethal outcome was 254.22 ± 28.5. This difference was statistically significant ($p<0,001$).

Early, biomarker based identification of neonates at risk of kidney injury is a fundamental step toward AKI prevention. NGAL has ability to predict AKI before clinical signs are evident and can facilitate implementation of appropriate preventive measures and improve resource utilization. Its use allows us to make the right clinical decisions at the right time, before the illness is clinically manifest and take appropriate measures to prevent renal function decline.

***Keywords*:** acute kidney injury, neonates, NGAL

Introduction

Acute kidney injury (AKI) is a serious problem in neonates in intensive care units (ICUs). It is defined as a rapid decrease in glomerular filtration that leads to retention of creatinine and nitrogenous waste products and usually with a decrease in urine output [1,2].

Acute kidney injury occurs in 2 to 8 % of adult patients hospitalized in ICU and the mortality rate is up to 50%. In children, incidence of AKI is significantly lower except in neonates. The incidence of AKI in neonates is 8 to 24% and the mortality rate ranges up to 35% (10-60%) [3-5].

Risk factors for development of kidney injury in neonates include very low birth weight (less than 1500 g), low APGAR score in 5-th minutes after birth, intubation at birth, invasive mechanical ventilation, drugs administration (nonsteroidal anti-inflammatory drugs, antibiotics), insertion of umbilical central line (UVC/UAC), cardiac surgery [6,7].

So far, the diagnosis of AKI has been based on the determination of serum creatinine (sCr) which is the most commonly used parameter of glomerular filtration. But its use in the neonatal period is significantly limited. During the first 2 to 3 days of life, serum creatinine reflects the function of maternal kidneys, showing higher values that subsequently decrease in the following days. On the other hand, the level of sCr may increase even after 48-72 hours of initial kidney injury. Therefore, sCr is considered as a late functional marker of AKI [8,9].

Renal biomarkers can detect the kidney injury in the first 2 to 3 hours of its occurrence, even before there is a decrease in urine output and an increase in sCr. After more than a decade of intensive research effort, several renal biomarkers have been identified, including neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), cystatin C, IL-18, and liver-type fatty acid–binding protein (L-FABP) [10-13].

In our study we have used NGAL because it represents the most promising biomarker for early detection of kidney injury. NGAL, also termed siderocalin or lipocalin 2, is a protease resistant 25-kDa polypeptide of the lipocalin superfamily, initially identified in human neutrophils. It is expressed in trace levels in various human epithelia including kidney, trachea, lungs and colon. It functions as a natural siderophore by scavenging the cellular and pericellular labile iron that is released from organelles during an ischemic or toxic insult [14].

NGAL is increase proportionally to the severity and duration of renal injury, is expressed very early after renal injury (within 1–3 h of renal insult), is obtained from a readily available source (urine or plasma), and can be rapidly estimate. Its ability to predict AKI before clinical signs are evident and can facilitate implementation of appropriate preventive measures and improve resource utilization [15-17].

The aim of the study was to determine the role of biomarker NGAL in early detection of kidney injury in neonates.

Methods

The study evaluated the neonates suffering kidney injury who at the period of three years were treated at the University Children's Hospital in Skopje. It was carried out at the NICU where the most of neonates with kidney injury were referred from all the centres from the territory of Republic of North Macedonia.

AKI was defined by progressive increase in the neonates's serum creatinine level higher than 130 umol/L in neonates younger than 33 weeks of gestation and higher than 90 umol/L in neonates older than 33 weeks of gestation. The presence of oliguria was defined as a urinary output less than 1.0 ml/kg/h. According to our criteria all neonates who had major congenital abnormalities and neonates who were under 25 weeks of gestation and older than 28 days of age were excluded from the study. Medical data records of admitted neonates with kidney injury were analyzed. The laboratory examinations of serum creatinine values were done in the biochemical laboratory of the Children's Hospital using Kodak camera dry biochemistry. All cases of neonates with kidney injury were analyzed according to the following criteria: gender, gestational age, birth weight and risk factors such as asphyxia, sepsis, prematurity, meconium plug syndrome and congenital heart diseases.

NGAL was analyzed in urine samples collected on two occasions (day 0, day of admission and 2 days later). The taken urinary samples were frozen at minus -70 to -80 degrees. They were then transferred to the Institute of Biochemistry and the concentration of NGAL in urinary samples was determined using NGAL ELISA KIT (Bioporto).

The data collected were statistically analyzed using the methods of descriptive statistics. To determine the significance of differences in the parameters, the tests for independent samples were analyzed. Statistical significance was determined for the values of $p<0.05$.

Results

The study was carried out at the neonatal intensive care unit at the University Children's Hospital Skopje in which neonates with documented acute kidney injury were evaluated. The whole study has been performed during the 3 year period.

The estimated prevalence of AKI in neonates was 6.4%. According to gender distribution, males neonates predominated in 68% compared to females in 32% of causes. The male to female ratio was 2.1: 1. Most of the neonates involved in the study were term born (62%). The average age of gestation was 37. 3 ± 34 weeks and the average birth weight were 2890. 80 ± 898 g. Demographic characteristics of neonates are summarized in Table 1.

	Mean ± SD	Min	max
Gestational age (wk)	37.34 ±3.01	26	40
Weight (gr)	2890.80±898.12	780	4850
Duration of stay (days)	12.82±8.42	5	28

Table 1. Demographic characteristics of hospitalized neonates with AKI

Figure 1 shows the distribution of predisposing factors associated with acute kidney injury among neonatal patients. Perinatal asphyxia was the most common predisposing factor observed in 30% of neonates. Other predisposing factors were: sepsis and prematurity observed in 24% of neonates, congenital heart diseases in 12 % and meconium plug syndrome in 10% of neonates.

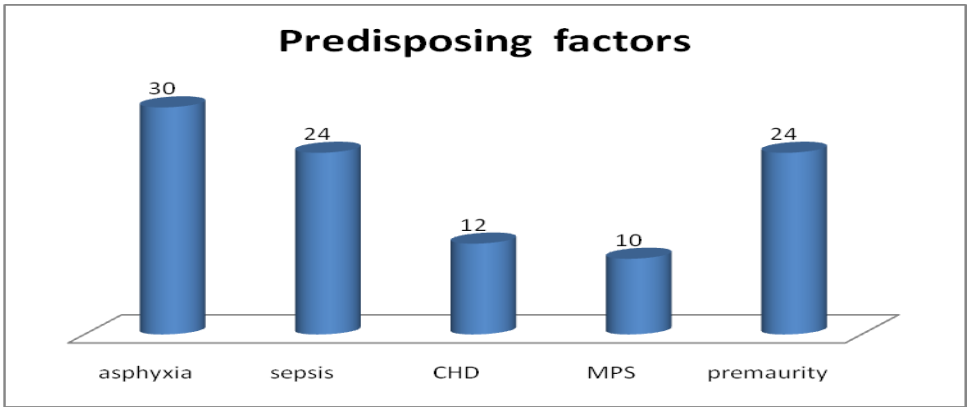

Figure 1. Predisposing factors associated with AKI

The mean serum creatinine (SCr) value in neonates with AKI was 76.78 ± 30.6 mmol / l at the day of admission to the NICU. While the value realized after 72 hours was 184.44 ± 103.74 mmol / l. In the next days SCr show decreasing trend. There were significant differences between sCr values during the study time (p<0,001). Distribution of sCr in neonates with AKI is shown on Figure 2.

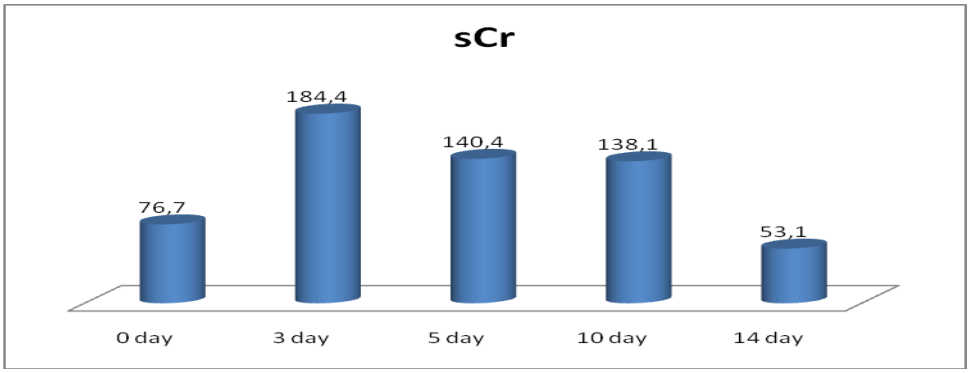

Figure 2. Distribution of sCr in neonates with AKI

The analyzed results showed a higher values of urinary NGAL in neonates with AKI on the day of admission (373.8 ± 194.9) and a slight upward trend, with further increase in the third day after admission (439.4 ± 254, 7). There was a significant difference between the uNGAL values and sCr values on the day of admission of neonates in NICU, p<0,001. Figure 3.

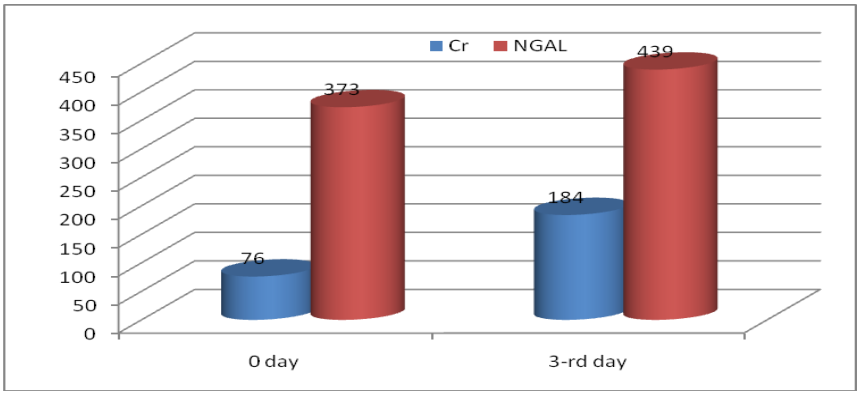

Figure 3. Distribution of creatinine and uNGAL values

There was a significant difference between the values of urinary NGAL in neonates with AKI and lethal outcome and neonates without lethal outcome (p<0,001). The mean urinary NGAL values in neonates with AKI and lethal outcome was 586.39 ± 182.3 while the mean values in neonates without lethal outcome was 254.22 ± 28.5, Figure 4.

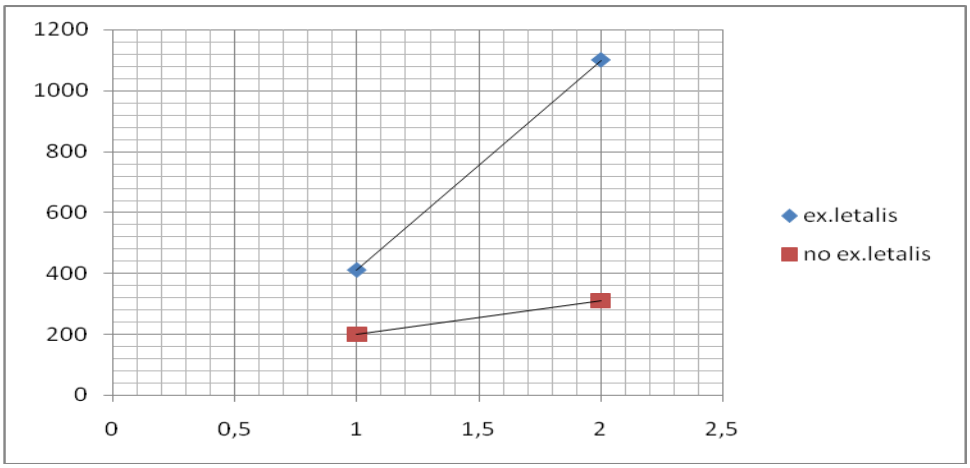

Figure 4. NGAL in neonates with AKI and lethal outcome compared to neonates without lethal outcome

Discussion

The study was carried out at the University Children's Hospital in Skopje where were referred most of the neonates with documented acute kidney injury from the territory of R.N.Macedonia.

The estimated prevalence of AKI in neonates was 6.4%, which correlate with findings in the literature, where the incidence of neonatal AKI was the highest incidence followed by adults and children, depending of different factors such as gestational age, birth weight, predisposing factors and

the facilities of the NICU. A similar finding has been published in the study of *Vachvanichsanong et al.* where the incidence of AKI in neonates was 6.3%, while in study of *Bolat et al.* was 8.0%. However, there are opposite finding. Thus, in the study of *Momtaz et al.* the incidence of AKI was 1.5%, in the study of *Mortazavi et al***.** 2.7% and at the *Agras et al.* was 3.4%. We assume that these differences can be due to differences in the criteria for diagnosing kidney injury in neonates. [18,19].

Perinatal asphyxia was the most common predisposing factors associated to AKI observed in 30% of neonates. *Abu-Haweleh et al*. and *Mortazavi et al.* published similar findings in their studies as well. Abu-Haweleh reports that 42% and Mortazavi reports that 30% of the patients with AKI have perinatal asphyxia as a dominant predisposing factors [20,21]. Different findings presented Kapoor *et al.* And *Youssef et al.* in their studies. Namely, Kapoor and Youssef found that neonatal sepsis was the commonest cause of AKI in 60% and 63% of cases of neonatal AKI [22-24].

NGAL as an early biomarker of AKI in neonates was first applied in this study. Before the use of biomarker, the diagnosis of kidney injury was based on determination of serum creatinine. In the study we find an increasing value of serum creatinine 72 hours after hospitalization of neonates at the NICU. This finding shows that by measuring serum creatine, the disease can be detected when clear clinical signs and symptoms are already present. This confirms that serum creatinine is a late functional marker of kidney injury [25,26].

In the study we have detected significantly higher values of urinary NGAL on the day of admission of critically ill neonates at the NICU compared to creatinine values. This finding indicates that the NGAL is a sensitive marker in the diagnosis of kidney injury. This data correlates with the data presented in the studies of *Nickolas et al*, and *Youssef et al*, emphasizing the role of the NGAL as a sensitive marker in detecting kidney injury [27,28].

Follow-up of urinary NGAL, on the day of hospitalization of neonates at the NICU, and three days later, showed a growing trend of an average value. The finding of significantly higher NGAL values at the first measurement suggests that renal damage is probably done before the hospitalization of neonates at the NICU. Namely, all critically ill neonates from the territory of the Republic of North Macedonia refer to our department, which is the only, specialized, tertiary center for intensive therapy. This explains the presence of kidney injury when receiving critically ill neonates, in which AKI is not yet clinically manifested. On the other hand, the high values of urinary NGAL registered on admission date in NICU, confirm the role of NGAL as an early marker of kidney injury. *Mishra et al,* and *Devarajan et al*., in their studies also refer a NGAL's ability to early detection of neonates with risk of AKI [29].

NGAL values in neonates with AKI and lethal outcomes were significantly higher than in neonates without lethal outcome. This finding suggests that the level of urinary NGAL significantly correlates with the severity and outcome of the disease, indicating the extent of current kidney injury. *Zappitelli et al*, in his study reported a similar results of significantly higher NGAL values in critically ill children with AKI and lethal outcomes [30].

The findings in our study confirm the applicability of the NGAL biomarker in the early diagnosis of acute kidney injury in the neonates.

Conclusion

Early, biomarker-based identification of neonates at risk of kidney injury is a fundamental step toward AKI prevention. The future of NGAL biomarker remains exciting, because it could provide early diagnosis of kidney injury in the first hours of its occurrence. Its ability to predict AKI before clinical signs are evident and can facilitate implementation of appropriate preventive measures and improve resource utilization. Its use allows us to make the right clinical decisions at the right time, before the illness is clinically manifest and take appropriate measures to prevent renal function decline. More research is needed to validate the benefits and cost-effectiveness of using NGAL for early implementation of nephroprotective strategies in neonates at risk of AKI.

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