Does high protein intake cause tubular injury in very preterm neonates?

Henny Adriani Puspitasari (✉ henny.adriani01@ui.ac.id)

Universitas Indonesia

Partini Pudjiastuti Trihono

Universitas Indonesia

Pustika Amalia Wahidiyat

Universitas Indonesia

Research Article

Keywords: very preterm neonates, high protein intake, urinary neutrophil gelatinase-associated lipocalin

Posted Date: June 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-136265/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Very preterm birth rate was 10.8% of all preterm in Asia. Early aggressive nutritional strategies in very preterm neonates is important for catching up growth; however, preterm kidneys have fewer, immature functional nephrons. Studies have showed that high protein intake induces nephron hypertrophy, proteinuria, and glomerular sclerosis through single nephron glomerular hyperfiltration (SNGHF), which leads to glomerulotubular injury.

Aim: to analyse the correlation between protein intake and glomerulotubular injury in very preterm neonates.

Method: A prospective cohort study was conducted in neonatal units of two hospitals in Jakarta. Urine samples were taken three times at post-natal ages 0-48 hours (T1), 72 hours (T2), and 21 days (T3) for determining the urinary neutrophil gelatinase-associated lipocalin to creatinine (uNGAL/Cr) ratio. Protein intake were given in accordance with local guideline while considering the clinical condition of participants. Protein levels from formula milk were recorded daily from 14-21 days of age, while breastmilk protein was measured twice by using a human milk analyser. Urinary NGAL (uNGAL) was tested with an ELISA. Glomerulotubular injury was defined as a uNGAL/Cr ratio ≥ 1 SD (22.74 ng/mg) at post-natal age 21 days. High protein intake was defined as average protein intake ≥ 3 g/kg/day.

Results: Fifty-nine very preterm neonates were recruited, of which 39 completed the study. Glomerulotubular injury was found in 9 of 39 participants (23%). The proportion of glomerulotubular injury in very preterm neonates who had received high protein intake vs low protein intake was 5 of 29 vs 4 of 10 participants, respectively. The median of uNGAL/Cr ratio was not significantly different in the high vs low protein intake group (3.54 (range: 0.69-89.16) ng/mg vs (6.88 (range: 0.32-66.64)) ng/mg, respectively. The uNGAL/C ratio was not correlated with protein intake. However, it was inversely correlated with gestational age and birth weight.

Conclusions: The proportion of glomerulotubular injury in very preterm neonates given high protein diet was 5 of 29. The uNGAL/Cr ratio was increased at the post-natal age of 72 hours and decreased in 21 days in both high and low protein intake groups. High protein intake was not correlated with glomerulotubular injury.

Introduction

Indonesia has the fifth highest rate of preterm birth in the world. In 2014, the preterm birth rate was 10.4%.¹ Data from a tertiary referral hospital in Jakarta reported that the absolute preterm birth rate in 2018 was 507, of which 112 were very preterm. Very preterm neonates (VPN) are defined as infants born at 28-32 weeks of gestational age; the survival rate in our centre was 58.9%.² The survival rates of VPN have been increasing as advancements in neonatology care continue to be made. Hence, morbidity caused by organ dysfunction is also increasing, including kidney dysfunction.

Very preterm neonates are born with a low number of functional nephrons, which results in a decrease in glomerular filtration surface area and an increase in glomerular arterial pressure.^{3,4} This mechanism increases the risk of hypertension and chronic kidney disease (CKD) later in life.⁴ Moreover, tubular immaturity, dysfunction of glomerular vasoregulation, low kidney perfusion, renal thrombosis, exposure to nephrotoxic agents and other events leading to acute kidney injury (AKI) are often found during the care of VPN.⁵ It should be remembered that AKI is a clinical term, whilst pathology findings describe it as acute tubular injury (ATI). Clinically, AKI is defined as an abrupt increase in serum creatinine level and/or decrease in urine production. Histopathologically, AKI was shown as focal or diffuse tubular luminal dilatation, simplification of the lining epithelium, loss of the brush border in proximal tubules, loss of nuclei and/or the presence of nucleoli. Proximal and distal tubules may be affected by AKI.⁶ Acute kidney injury affected 48% of 22 - <29 weeks of gestational age preterm neonates and 18% of 29 - <36 weeks of gestational age preterm neonates participate in the AWAKEN cohort study.⁷

Early tubular injury during AKI do not cause an increase in serum creatinine level but detectable by some biomarkers, such as urinary neutrophil gelatinase-associated lipocalin (uNGAL).⁸ Expression of uNGAL increases due to decrease reabsorption of NGAL during proximal tubular cells injury. Moreover, *de novo* synthesis of NGAL increases during AKI in the distal nephron segment, especially thick ascending limbs of Henle and collective ducts, which contributes to the largest fraction of urinary NGAL. A non-invasive method of obtaining urine samples used in uNGAL examination is beneficial for VPN since blood sampling is highly restricted due to their low blood volume. In order to control the dilutional factor in urine upon uNGAL testing, normalisation by using the uNGAL to creatinine (uNGAL/Cr ratio) is commonly performed.⁹ In preterm neonates, uNGAL/Cr cut off more than 100 ng/mg predicts AKI with 89% sensitivity and 33% specificity.¹⁰ Another study in neonates born ≥ 1200 g or ≥ 31 weeks of GA reported higher median of uNGAL/Cr level in AKI group (6.11×10^6 [range: $3.38 \times 10^6 - 18.9 \times 10^6$] pg/mg) compare to non-AKI group (3.17×10^6 [range: $1.59 \times 10^6 - 6.08 \times 10^6$] pg/mg; $p=0.003$).¹¹

Aggressive nutritional intervention by administering a high protein diet has been used in our NICU to accelerate growth and improve the neurodevelopmental outcomes of VPN.¹² This strategy is started by administering 2.5 g/kg/day protein soon after birth and gradually increasing to 4 g/kg/day by post-natal age day 3.¹³ Experimental animal and human studies showed that a high protein diet induces kidney hypertrophy, proteinuria, and glomerulosclerosis via single nephron glomerular hyperfiltration (SNGHF). Hypothetically, SNGHF is an early developmental process of chronic kidney disease in adulthood.¹⁴ Since preterm neonates need high protein intake to catch up to healthy growth percentiles, the impact of high protein intake on preterm nephrons as well as the potential long term effects of this diet requires additional research. A previous study reported that very preterm infants receiving a protein diet of up to 7 g/kg/day showed an increase in the estimated glomerular filtration rate (eGFR).¹⁵ However, another study showed that protein intake of up to 3.6 g/kg/day in very preterm infants did not increase the levels of urea, creatinine, cystatin C serum and urinary b2 microglobulin.¹⁶ The purpose of this study was to examine the correlation between protein intake and tubular injury in VPN.

Methods

Study population

This study was a prospective cohort study from birth to 21 days of age involving 59 VPN admitted to the NICU of two referral hospitals, Cipto Mangunkusumo General Hospital (CMGH) and Bunda Menteng Hospital (BMH), between June 2019 and May 2020. Participants were recruited consecutively and excluded if there were major congenital abnormalities, intrauterine growth restriction (IUGR), maternal exposure to nephrotoxic drugs during pregnancy, difficulties in collecting urine samples, and parental refusal of consent to participate in the study. Sample size was calculated based on coefficient correlation formula with 20% drop outs was estimated.¹⁷ The sample size estimation was 39 subjects.

This study measured tubular injury, protein intake, and other variables. The primary outcome was the correlation between protein intake and tubular injury. Protein intake level, prevalence of tubular injury, weight increment, weight to age z-score (WAZ), comorbidities, and factors associated with tubular injury were secondary outcomes measured in this study.

Tubular injury

Spot urine samples were collected three times during the study, at post-natal age 0-48 hours (T1), 72 hours (T2), and 21 days (T3). At least 5 mL of urine was collected each time with a urine collector or urethral catheter for uNGAL and urine creatinine. Samples were kept in a refrigerator at a temperature of 13-15°C for a maximum of 12 hours and then were sent to Prodia Laboratory in a cooler box at the same temperature. Samples were centrifuged, and some were tested randomly for urine creatinine (uCr). Supernatants were then frozen at -70°C until further analysis. Urinary NGAL (uNGAL) was tested with an ELISA method using QUANTIKINE Immunoassay (R&D Systems, Minneapolis, USA; NGAL Immunoassay). The urinary NGAL to creatinine ratio was produced by dividing uNGAL (ng/mL) by uCr (mg/dL). The distribution of the uNGAL/Cr ratio was statistically analysed, and the standard deviation was determined. Tubular injury was defined as a spot urinary NGAL to creatinine (uNGAL/Cr) ratio ≥ 1 SD at day 21.

Protein intake

Protein intake included the total parenteral protein in the form of amino acids and oral/enteral protein found in breastmilk or formula milk administered to the participants. The protein intake level was administered according to NICU guidelines for VPN, which were 2.5 g/kg/day of protein in the first 24 hours; 3.5 g/kg/day in the next 24 hours; and 4 g/kg/day thereafter. Neonates were then divided into two groups based on protein intake. Low protein intake was defined as <3 g/kg/day and high protein intake was defined as ≥ 3 g/kg/day. Protein intake was recorded from the medical chart at 0-48 hours, 72 hours, and 21 days. From 14 to 21 days of age, the total fluid and total protein intake from both parenteral and enteral sources were recorded. Full feed was defined as consumption of at least 100 mL/kg body weight/day of milk orally/enterally. Enteral diet sources included breast milk, formula milk, and fortified breast milk. Protein intake from formula milk was calculated by dividing the total protein in 100 mL of

milk and body weight. Protein intake from breastmilk was calculated by taking the average breastmilk protein content at day 14 and 21. The protein content of breastmilk was measured by sampling 5 mL of breastmilk and testing it in a human milk analyser (MIRIS, Uppsala, Sweden; infrared spectroscopy). Protein content from a human milk fortifier (HMF) was also measured based on the volume added to the breastmilk.

Other variables

Data from participants who completed the study up to 21 days were recorded for prenatal dan perinatal events. Prenatal data on maternal infection, hypertension, diabetes mellitus, anemia and hemoglobin level were recorded. Birth data including birth length, birth weight, head circumference, APGAR scores 1 and 5 minutes were recorded. Weight increments were monitored at day 14 and 21 and plotted to an INTERGROWTH post-natal growth (IPNG) curve, producing a weight to age z-score (WAZ). During hospitalisation, secondary data of perinatal factors from medical records were collected. Those data consisted of sepsis, respiratory distress, the use and duration of invasive mechanical ventilation (IMV), non-invasive ventilation (NIV); vasoactive medications; diuretics, nephrotoxic medication exposure, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), and intraventricular haemorrhage (IVH).

Statistical analysis

Clinical characteristics and other variables were described by the median and range and the mean and standard deviation. Correlation of protein intake and the uNGAL/Cr ratio at T1, T2 and T3 were analysed with Spearman's analysis. A chi square test was performed to examine the association between protein intake and Tubular injury. The correlation of birth weight with the uNGAL/Cr ratio was then analysed with Spearman's test. A bivariate analysis was performed to describe factors associated with tubular injury. A Fisher's exact test was carried out to assess association between tubular injury and maternal factors (infection, hypertension, diabetes, anemia), nephrotoxic medication exposure, sepsis, the use of IMV; NIV; vasoactive medications and diuretics. An independent t test was performed for birth weight, gestational age and mean protein intake associated with tubular injury. Mann Whitney test was performed for APGAR score, duration of IMV; NIV; vasoactive medication use; diuretic use, and maternal hemoglobin level associated with tubular injury. All factors with significancy level ≤ 0.05 on bivariate analysis were analysed by logistic regression model as multivariate analysis. Statistical analysis was performed using SPSS version 17 (IBM Corp. Armonk, NY, IBM Corp). This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia under the protocol number KET-400/UN2.F1/ETIK/PPM.00.02/2019.

Results

Urine samples were collected consecutively from 59 VPN. Thirty-nine (66.1%) participants completed the cohort study but 20 (33.9%) participants died as described in **Figure 1**. One hundred and fifty-one urine samples were available for analysis.

Participant characteristics are shown in **Table 1**. Data for nephrotoxic medication exposure and comorbidities were only recorded for participants at CMGH. Echocardiography and head ultrasound were performed to 44 and 41 participants. The median protein intake at the age of 0-48 hours, 72 hours, and 21 days were 2.09 (range: 0.72-3.48), 3.12 (range: 0.87-4), and 3.44 (range: 2-5.18) g/kg/day, respectively. The median uNGAL/Cr ratios at post-natal age 0-48 hours, 72 hours, and 21 days were 7.75 (range: 0.45-104.11), 12.58 (range: 0.39- 63.78), 4.22 (range: 0.32-89.16) ng/mg, respectively. We found no difference in uNGAL/Cr ratios between the high protein intake group and the low protein intake group across post-natal ages 0-48-hours, 72 hours, and 21 days (**Table 2**).

Table 1. Participant characteristics

Characteristics		N	
Birth weight (g)	mean (SD)	59	1236.2 (237.1)
Gestational age (weeks)	median (range)	59	30 (28-32)
Weight increment (g/kg/day)	median (range)	59	
Post-natal age 0-14 days		45	0 (-13 – 3.2)
Post-natal age 0-21 days		59	6.5 (-95.4 – 62.9)
Weight to age z-score (WAZ)	median (range)	59	
WAZ at birth		59	0.3 (-2.5 – 3.6)
WAZ at post-natal age 21 days		59	1.3 (-2.9 – 2.8)
Sex	n (%)	59	
Male		37	37 (62.7)
Female		22	22 (37.3)
Nephrotoxic medication	n (%)	56	44 (78.6)
Necrotizing enterocolitis/NEC	n (%)	56	33 (58.9)
Patent ductus arteriosus/PDA	n (%)	44	19 (43.2)
Intraventricular haemorrhage/IVH	n (%)	41	13 (31.7)

Table 2. uNGAL/Cr level in relation to protein intake

Time	uNGAL/Cr level (ng/mg)		p
	Low protein intake (n=11) median (range)	High protein intake (n=28) median (range)	
T1	9.06 (0.45-39.27)	11.40 (0.63-104.11)	0.62
T2	19,79 (0.97-45,70)	12.47 (039-63.78)	0.38
T3	6,88 (0.32-66.64)	3.54 (0.6-89.16)	0.30

T1: post-natal age 0-48 hours; T2: 72 hours; T3: 21 days

Protein intake and tubular injury

The uNGAL/Cr were not related to protein intake at all time of examination as shown in **Figure 2**. An association between protein intake and tubular injury is shown on **Table 3**. Tubular injury was defined as a uNGAL/Cr ratio above 1 SD (22.74 ng/mg) at post-natal age 21 days. The prevalence of tubular injury in VPN was 9/39 (23%). In the high protein intake group, 5 of 29 participants had tubular injury. In addition, 4 of 10 participants had tubular injuries in the low protein intake group. The prevalence of tubular injury in both groups was not statistically different ($p=0.2$).

Table 3. Association of protein intake and glomerulotubular injury

	Glomerulotubular injury		Total
	No, n (%)	Yes, n (%)	
Protein intake			
Low (<3 g/kg/day)	6 (60%)	4 (40%)	10
High (≥3 g/kg/day)	24 (82.8%)	5 (17.2%)	29

Factors associated with tubular injury

Prenatal dan perinatal factors related to tubular injury at the age of 21 days were described in **Table 4**. Data of prenatal and perinatal factors were recorded from 39 patients. Maternal history data were completed in 38 participants, while sepsis and nephrotoxic medication data were obtained from 36 participants. Birth weight was inversely correlated with the uNGAL/Cr ratio at post-natal age 0-48 hours ($r=-0.31$, $p=0.016$), 72 hours ($r=-0.42$, $p=0.002$) and 21 days ($r=-0.36$, $p=0.025$). From 38 data collected on maternal history, we found that maternal infection, hypertension, diabetes, and anemia were found respectively in 16 (42.1%), 10 (26.3%), 1 (2.6%), and 20 (52.6%) in neonates who had tubular injury. Since our hospital was a tertiary hospital, most were referral cases with no prior data of antenatal ultrasound. However, any clinical issues regarding congenital anomaly of kidney and urinary tract found after delivery were consulted to pediatric nephrologist as per protocol. No pediatric nephrologist consultations were received from participants. Sepsis, nephrotoxic medication, and RD were recorded in 26 of 36 participants (72.2%), 36 of 39 participants (92.3%), and 35 of 39 participants (89.7%) in tubular injury group, respectively. Ventilation support were given by IMV in 18 (46%) participants and NIV in 30 (76.9%) participants with RD who had tubular injury.

Table 4. Prenatal and perinatal factors associated with glomerulotubular injury at 21 days of age

Factors		Glomerulotubular injury		p value
		(N=39)		
		Yes	No	
Pre-natal factors				
Birth weight (g)	mean	1160.63	1308.03	0.05
	(SD)	(156.1)	(243.7)	
Gestational age (weeks)	mean	29.75 (2.1)	30.26 (1.9)	0.54
	(SD)			
APGAR 1 minutes score,	mean	2.13 (3.1)	3.77 (3.2)	0.57
	(SD)			
APGAR 5 minutes score,	mean	4.38 (4.7)	5.42 (3.9)	0.94
	(SD)			
Maternal infection (N=38)	n (%)	16 (42.1)	22 (57.9)	1.00
Maternal hypertension (N=38)	n (%)	10 (26.3)	28 (73.7)	0.67
Maternal diabetes (N=38)	n (%)	1 (2.6)	37 (97.3)	0.28
Maternal anaemia (N=38)	n (%)	20 (52.6)	18 (47.3)	0.25
Maternal haemoglobin level (g/dL)	mean	7.36 (7.17)	6.62 (8.41)	0.81
(N=38)	(SD)			
Perinatal factors				
Sepsis (N=36)	n (%)	26 (72.2)	10 (27.8)	0.56
Respiratory distress	n (%)	35 (89.7)	4 (10.3)	1.00
Invasive mechanical ventilation	n (%)	18 (46.2)	21 (53.8)	0.71
(IMV)				
Duration of IMV (days)	mean	7.50 (8.60)	4.32 (6.81)	
	(SD)			
Non-invasive ventilation (NIV)	n (%)	30 (76.9)	9 (23.1)	1.00
Duration of NIV (days)	mean	6.0 (5.6)	8.5 (8.8)	0.96
	(SD)			
Vasoactive medication	n (%)	7 (17.9)	32 (7.7)	0.32
Nephrotoxic medication	n (%)	36 (92.3)	3 (7.7)	1.00
Diuretics	n (%)	3 (7.7)	36 (92.3)	0.13

Protein intake 0-48 hours (g/kg/day))	mean (SD)	1.96 (0.82)	1.98 (0.69)	0.96
Protein intake 72 hours (g/kg/day)	mean (SD)	2.96 (0.35)	2.99 (0.58)	0.85
Protein intake 21 days (g/kg/day)	mean (SD)	3.03 (0.47)	3.67 (0.79)	0.01

Birth weight, maternal anemia, use of diuretics, and mean protein intake at 21 days were different among tubular injury group and non-tubular injury group. We performed multivariate analysis on those factors and found that the only factor associated with tubular injury was mean protein intake at 21 days ($p=0.01$).

Discussion

Our study found no correlation between protein intake and uNGAL/Cr ratios as a biomarker for tubular injury at post-natal age 48 hours, 72 hours, and 21 days. This correlation was illustrated in **Figure 2**. In the first 48 hours of life, some VPN received a protein level that was lower compared to the guideline, with a median of 2.09 (range: 0.72-3.48) g protein administered per day. This protein intake level was not significantly correlated with the uNGAL/Cr ratio. However, the pattern in **Figure 2A** shows that the uNGAL/Cr ratio increased proportionally with the increase in protein intake. During the first 48 hours, VPN experience physiological and haemodynamic stress in their new extra-uterine environment, which impacts uNGAL/Cr ratio. Furthermore, tubular immaturity due to immature resorptive function during the early neonatal period causes an increase of the uNGAL/Cr ratio along with an increase in protein intake.

In contrast to post-natal age 0-48 hours, there was no significant correlation between protein intake and tubular injury at post-natal age 72 hours and 21 days. At these points, the higher the protein intake, the lower the uNGAL/Cr ratio was (**Figure 2B and 2C**). Tubular maturation process that occurs with age contributes to the improvement of tubular resorptive function of uNGAL.

Our results support previous studies which also showed no impairment of tubules and glomeruli in very preterm infants with protein intake levels up to 3.6 g/kg/day at the age of 14 days.¹⁶ This study measured serum urea, creatinine, cystatin C and urinary b2 microglobulin as biomarkers for glomerular and tubular injury. The protein intake level given to neonates in our study at post-natal age 0-48 hours, 72 hours, and 21 days were in the range of the study, which was 3 to 3.6 g/kg/day.¹⁶ Another study reported a decrease in eGFR, sodium clearance, and osmolality with the administration of breastmilk with supplemental protein up to 7 g.¹⁵

Traditional AKI definition by serum creatinine and urine production criteria detects AKI only after at least 50% injury of the nephron occurs. Creatinine was influenced by maternal serum creatinine level, muscle mass, hydration states, and sex.¹⁸ During AKI, tubular injury was occurred first before increase in serum creatinine. Biomarkers were developed to detect the early tubular injury so that AKI can be detected and managed earlier. These biomarkers can be detected in the blood and/or urine. Serum cystatin C increases after AKI in neonates, but around 2 mL of plasma volume needs to be drawn from tiny neonates. Another urinary biomarker such as uNGAL, urine b2 microglobulin, KIM-1, urine LFABP were studied in pretermneonates for early detection of kidney injury. These biomarkers were taken from urin sample which easier for VPN.¹⁹ Tubular injury in our study was defined as uNGAL/Cr level ≥ 1 SD at day 21. We found that the proportion of VPN experiencing tubular injury was 9/23 (23%) with no difference between the high protein intake group and the low protein intake group. Tubular injury occurred in 5 of 9 participants receiving high protein intake (>3 g/kg/day) and in 4 of 10 subjects receiving low protein intake (≤ 3 g/kg/day). Serum creatinine are measured as per indication, and not part of this study due to those reasons. Thus, the prevalence of AKI was similar to the prevalence observed in a previous study involving 2,110 preterm neonates in the USA. Acute kidney injury prevalence was 28% in neonates at a gestational age of 22-28 weeks and 14% in neonates at a gestational age of 29-35 weeks based on the modified KDIGO criteria. Acute kidney injury in VPN was associated with an increased risk of death (adjusted odds ratio (OR) 2.8; 95% CI 1.7-4.7) and prolonged hospitalisation. Factors found to be associated with AKI in very preterm birth were birth outside the hospital, the use of epinephrine in resuscitation, hyperbilirubinemia on admission, hereditary metabolic diseases, surgical intervention, more frequent renal function surveillance and admission to a paediatrics hospital.²⁰ Those factors were not addressed in our study.

All participants who had tubular injury in both intake groups were exposed to nephrotoxic antibiotics, gentamicin and/or amikacin. Four participants in the low protein intake group who experienced tubular injury received two types of nephrotoxic drugs, gentamicin and amikacin, during hospitalisation. Gentamicin and amikacin cause tubular injury by damaging segment S1 and S2 (proximal tubules), whilst NGAL is mostly produced as a result of damage of the proximal tubule.²¹ The “baby NINJA” study reported positive associations between the number of nephrotoxic agents administered in the NICU and the increase risk of AKI in very preterm infants.²² Nonetheless, bivariate analysis of several factors such as nephrotoxic drug exposure, sepsis, gestational age and birth weight showed no association with tubular injury at the age of 21 days in our participants. However, it is also important to mention that this study was not designed to analyse those factors, and the number of participants may have affected the results of the statistical analysis.

Importantly, the uNGAL/Cr ratio was affected by gestational age and birth weight. We found that the uNGAL/Cr ratio was inversely related to gestational age in VPN. As reported in the previous study, gestational age is the only parameter affecting the uNGAL/Cr ratio in the mixed model regression analysis compared with other parameters, such as sex, race, prevalence of AKI, and the insertion of an umbilical artery catheter.²³ In the first 48 hours, participants with higher gestational ages showed lower

uNGAL/Cr ratio baseline levels. In VPN, gestational age is a critical factor that affects the quantity of functional nephrons, the ability of the nephrons to filtrate and the maturation of renal tubules.²⁴ In contrast, the uNGAL/Cr ratio has been negatively correlated with birthweight.^{23,25} A significant correlation between a decrease in the uNGAL/Cr ratio and an increase in birthweight was shown by our study. Multivariate regression analysis at a logarithmic scale of the uNGAL/Cr ratio showed a significant effect of birthweight, gestational age, and post-natal age on uNGAL/Cr ratio. The level of uNGAL/Cr ratio decreases by 13.4% with a 100 g increase in birthweight (95% CI: -16,6;(-10,1)), and increases by 11.3% with a 1 week increase in gestational age (95% CI: -15,3; (-7,1)). Nonetheless, uNGAL/Cr ratio increases by 4.4% with a 1 day increase in post-natal age (95% CI: -5,6; (-3,2)).²⁶

We also found that the median protein intake received by participants at post-natal age 0-48 hours (2 (range: 0.72- 3.48)) g/kg/day, 72 hours (3.12 (range: 0.87- 4)) g/kg/day, and 21 days (3.44 (range: 2 – 5.18)) g/kg/day were lower than our NICU guidelines. Even though the protein intake was lower than expected, the weight to age z-score (WAZ) at 21 days post-natal age according to the IPNG curve showed that 32 of 39 VPN had an increase in body weight compared to their birth weight. This supports a previous systematic review that found that 3 g/kg/day protein administration is needed for making up growth in VPN.²⁷

The main strength of our study is the adequate number of samples used to show the correlation between protein intake and tubular injury. We also included only VPN with appropriate gestational ages, which excludes the confounding effect of low birth weight on tubular injury. Some limitations of this study include that the cut-off point of the uNGAL/Cr ratio to determine the tubular injury was based on the standard deviation of our data. Urine samples were not collected on a daily basis; therefore, variation in the post-natal period could have been missed in this study. We were unable to control the exposure to nephrotoxic drugs and sepsis in the VPN during our study observation. However, we included those factors in our analysis.

Conclusion

In conclusion, the present study shows no correlation between high protein intake and tubular injury in VPN. The proportion of tubular injury experienced by VPN in the high protein diet group was 5 of 29. In VPN, the uNGAL/Cr ratio was increased at 72 hours of age and decreased in 21 days in both the high and low protein intake groups. Our findings also support the current nutritional guideline for VPN, which recommends administering high protein levels to promote growth. A longer follow-up period and combination of tubular injury biomarkers should be studied further to monitor the long-term effects of high protein intake. In addition, other factors that contribute to tubular injury in VPN should be further evaluated.

Sample Size Estimation

Sample size was estimated by formula for correlation¹⁷:

$$n = \left[\frac{(Z_{\alpha} + Z_{\beta})}{0,5 \ln \left[\frac{(1+r)}{(1-r)} \right]} \right]^2 + 3$$

$$n = \left[\frac{(1,96 + 1,64)}{0,5 \ln \left[\frac{1+0,59}{1-0,59} \right]} \right]^2 + 3$$

$$n = 31$$

n = sample size

α = type I error, Zα = 1,96

b= type II error, 10%

r = correlation (0,59)²⁸

List Of Abbreviations

AKI	:	acute kidney injury
BMH	:	Bunda Menteng Hospital
CKD	:	chronic kidney disease
CMGH	:	Cipto Mangunkusumo General Hospital
eGFR	:	estimated glomerular filtration rate
IPNG	:	INTERGROWTH post-natal growth
IUGR	:	intrauterine growth restriction
IVH	:	intraventricular hemorrhage
NEC	:	necrotizing enterocolitis
NICU	:	neonatal intensive care unit
PDA	:	patent ductus arteriosus
SD	:	standard deviation
SNGHF	:	single nephron glomerular hyperfiltration
uCr	:	urine creatinine
uNGAL	:	urine neutrophil gelatinase-associated lipocalin
uNGAL/Cr	:	urine neutrophil gelatinase-associated lipocalin to creatinine ratio
VPN	:	Very preterm neonates
WAZ	:	weight to age z-score

Declarations

Acknowledgements

The authors would like to thank the NICU staff and nurses of CMGH and BMH for their cooperation in collecting samples for the study. We also thank Dr. Yoga Devaera, Sp.A(K) for her substantial contribution to the nutritional interpretation of the data; Dr. Rosalina D Roeslani, Sp.A(K) for her contribution to the neonatology aspect of the study protocol; Dr. Dejandra Rasnaya for the data collection; and Dr. Sudung O Pardede, Sp.A(K) and Dr. Ninik Asmaningsih, Sp.A(K) for their critical evaluation of the discussion.

Disclosure

This study was partially supported by a *Publikasi Terindeks Internasional Sains dan Teknologi Kesehatan* (PUTI Saintekes) research grant from the University of Indonesia, Indonesia. Grant number: NKB-

Author contributions

The authors made the following contributions: conception and design of the study (HAP, PPT), data collection (HAP), statistical analysis (HAP), interpretation of the data (HAP, PPT, PAW), article writing (HAP) and manuscript review and modification (HAP, PPT, PAW). All authors read and approved the final version of the manuscript.

References

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Heal*. 2019;7:e37–46.
2. Divisi Perinatologi Departemen Ilmu Kesehatan Anak FKUI RSCM. *Profil Data Perinatologi Rumah Sakit Cipto Mangunkusumo*. Jakarta; 2018.
3. Stritzke A, Thomas S, Amin H, Fusch C, Lodha A. Renal consequences of preterm birth. *Mol Cell Pediatr*. 2017;4:2.
4. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens*. 1988;1:335–47.
5. Chaturvedi S, Ng KH, Mammen C. The path to chronic kidney disease following acute kidney injury: a neonatal perspective. *Pediatr Nephrol*. 2017;32:227–41.
6. Gaut JP, Liapis H. Acute kidney injury pathology and pathophysiology: a retrospective review. *Clin Kidney J*. 2020;14:526–36.
7. Jetton JG, Guillet R, Askenazi DJ, Dill L, Jacobs J, Kent AL, et al. Assessment of worldwide acute kidney injury epidemiology in neonates: Design of a retrospective cohort study. *Front Pediatr*. 2016;4.
8. Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. *Biomark Med*. 2010;4:265–80.
9. Tang KWA, Toh QC, Teo BW. Normalisation of urinary biomarkers to creatinine for clinical practice and research – When and why. *Singapore Med J*. 2015;56:7–10.
10. DeFreitas MJ, Seeherunvong W, Katsoufis CP, RamachandraRao S, Duara S, Yasin S, et al. Longitudinal patterns of urine biomarkers in infants across gestational ages. *Pediatr Nephrol*. 2016;31:1179–88.

11. 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;11:1527–35.
12. Su B-H. Optimizing nutrition in preterm infants. *Pediatr Neonatol*. 2014;55:5–13.
13. Divisi Perinatologi Departemen Ilmu Kesehatan Anak FKUI RSCM. Standar Prosedur Operasional Neonatologi Rumah Sakit Cipto Mangunkusumo. Jakarta; 2019.
14. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med*. 1982;307:652–9.
15. Herin P, Zetterstrom R. Studies in Renal Response to Various Protein Intakes in Preterm Infants. *Acta Paediatr Scand*. 1987;76:447–52.
16. Kanmaz HG, Mutlu B, Erdevi O, Canpolat FE, Oguz SS, Uras N, et al. Does enteral protein intake affect renal glomerular and tubular functions in very low birth weight infants? *Clin Nephrol*. 2013;80:355–60.
17. Hulley S, Cummings S, Browner W, DG G, TB N. *Designing Clinical Research: an Epidemiologic Approach*, 4th Ed. Philadelphia: Lippincott Williams& Wilkins; 2013. p. 59-79.
18. Chen C-N, Chou C-H, Jeng S-F, Tsai I-J, Chen P-C, Chen C-Y, et al. Urinary Neutrophil Gelatinase-Associated Lipocalin Levels in Neonates. *Pediatr Neonatol*. 2016;57:207–12.
19. 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;11:1527–35.
20. Charlton JR, Boohaker L, Askenazi D, Brophy PD, D'Angio C, Fuloria M, et al. Incidence and risk factors of early onset neonatal AKI. *Clin J Am Soc Nephrol*. 2019;14:184–95.
21. Murphy HJ, Thomas B, Van Wyk B, Tierney SB, Selewski DT, Jetton JG. Nephrotoxic medications and acute kidney injury risk factors in the neonatal intensive care unit: clinical challenges for neonatologists and nephrologists. *Pediatr Nephrol*. 2019;
22. Stoops C, Stone S, Evans E, Dill L, Henderson T, Griffin R, et al. Baby NINJA (Nephrotoxic Injury Negated by Just-in-Time Action): Reduction of Nephrotoxic Medication-Associated Acute Kidney Injury in the Neonatal Intensive Care Unit. *J Pediatr*. 2019;
23. Askenazi DJ, Koralkar R, Levitan EB, Goldstein SL, Devarajan P, Khandrika S, et al. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. *Pediatr Res*. 2011;70:302–6.

24. Gubhaju L, Sutherland MR, Horne RSC, Medhurst A, Kent AL, Ramsden A, et al. Effect of fetal and child health on kidney development and long-term risk of hypertension and kidney disease. Clin Exp Nephrol. 2018;74:1–7.

25. Lavery AP, Meinzen-Derr JK, Anderson E, Ma Q, Bennett MR, Devarajan P, et al. Urinary NGAL in premature infants. Pediatr Res. 2008;64:423–8.

26. Miklaszewska M, Korohoda P, Drożdż D, Zachwieja K, Tomasik T, Moczulska A, et al. eGFR values and selected renal urine biomarkers in preterm neonates with uncomplicated clinical course. Adv Clin Exp Med. 2019;28:1657–66.

27. Fenton TR, Premji SS, Al-Wassia H, Sauve RS. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane database Syst Rev. 2014;CD003959.

28. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics. 2003;111:986–90.

Figures

Figure 1

Study population. A total of 59 participants were recruited. The first sample was collected at post-natal age 0-48 hours (T1). Of the 59 participants, 45 participants had a second sample collected at post-natal age 72 hours (T2), and 39 subjects had a third sample collected at post-natal age 21 days (T3) in both hospitals, Cipto Mangunkusumo General Hospital (CMGH) and Bunda Menteng Hospital (BMH).

Figure 2

The urine NGAL creatinine ratio was not correlated with protein intake at post-natal age 0-48 hours (T1), 72 hours (T2), and 21 days (T3). However, uNGAL/Cr was increased in protein intake at T1 (A) and decreased at T2 (B) and T3 (C).