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The Difference of Urinary Neutrophil Gelatinase-Associated Lipocalin Levels in Moderate Malnutrition and Well-Nutrition in School-age Children

Asri Rachmawati[*], Nanan Sekarwana, Susi Susanah

Department of Child Health, Faculty of Medicine, Universitas Padjadjaran/Hasan Sadikin General Hospital, Bandung. Indonesia
*Corresponding author: asri.gumelar23@gmail.com

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Abstract **Background.** The prevalence of school-age children with moderate malnutrition in Indonesia is high enough that growth monitoring and nutritional intervention in school-aged children are required. Moderate malnutrition in school-age children who are not well managed might reduce the quality of human resources in the productive age. Malnutrition can affects reduced protein synthesis, hypoalbuminemia, and decreased oncotic pressure, in addition to decrease in glomerular filtration rate and impaired tubular function. If the renal tubules damage, the level of Neutrophil gelatinase-associated lipocalin (NGAL) excreted in the urine will increase because NGAL can not be properly reabsorbed. NGAL is expressed by the tubules and passes through the urine quickly after the kidney is injured and inflamed. **Methodology/Principal Findings.** This was a cross-sectional study conducted from November-December 2017 in children aged 6–13 years old at Garuda Elementary School in Bandung. Subjects were selected through stratified random sampling. We got 39 children with moderate malnourished and 39 well-nourished children who met the study criterias. Weight, height and urine NGAL of subjects were measured. Nutritional status were determined using WHO Child Growth Standards. Urine sampling was done by collecting 10 ml of midstream urine in a sterile container, labeled with name, study registry number and date of material collection. The samples were then immediately sent to Hasan Sadikin Hospital laboratory. Urinary NGAL level was examined using ELISA method. Data analysis using Mann Whitney test. There was significant difference in urinary NGAL levels between well-nourished and moderate malnourished children ($p<0.05$). Median of urinary NGAL levels in moderate malnourished group were higher than in the well-nourished group with a difference of 43.6 and 95% CI between 38.3-50.1. **Conclusions.** The level of urinary NGAL (uNGAL) in moderately malnourished children were higher than in well-nourished children.

Keywords: *moderate malnutrtion, children, urinary NGAL*

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1. Introduction

The prevalence of school-age children with moderate malnutrition in Indonesia is high enough that growth monitoring and nutritional intervention in school-aged children is required. Based on WHO data in 2010, 55 million pre-school age children were malnourished, 40 million of whom had acute moderate malnutrition. According to data from Riset Kesehatan Dasar (Riskesdas) of the Ministry of Health of the Republic of Indonesia in 2013, 19.6% of under five children in Indonesia sufferred from moderate and severe malnutrition. While the prevalence of moderate malnutrition in primary school children in 2007 increased to 12.1% and decreased in the year 2013 to 11.2% [1,2,3,4,5]. Globally, in 2011, more than 100 million children aged less than 5 years (16%) had moderate malnutrition based on the WCGS curve. This percentage reduced by 36% from 159 million moderate malnutrition children in 1990. In China, the prevalence of moderate malnutrition in children aged 7-12 years was 21%, in Nigeria, the prevalence was 26.7% in adolescents, while in Bangladesh the prevalence was 84% [6,7]. In this research, the prevalence of moderate and severe malnutrition in Garuda Elementary School was 7%. This high prevalence indicates the importance of growth monitoring not only in toddlers but also in school-age children, as moderate malnutrition in school-age children who are not well managed can reduce the quality of human resources in productive ages.

Malnutrition contributes to 56% of child deaths in the world, and 83% of them are associated with moderate and severe malnutrition [3]. Moderate malnutrition in children have many health problems due to the lack of nutrients to the entire organ system which is the body's adaptations to reduce functional requirements in order to survive. Lack of nutrition affects renal function in the form of decreased glomerular filtration rate (GFR), sodium and phosphate excretion, and causes lower erythropoietin synthesis. Glomerular filtration rate decreases in malnourished children and improves when nutritional status returns well [4,8,9,10].

In the state of undernutrition, there is a decrease in protein synthesis, hypoalbuminemia and reduced oncotic pressure [11]. Benabe et al. stated that the lack of protein intake is associated with increased angiotensinogen mRNA in adipocyte perivascular and ACE mRNA in endothelial cells of intrarenal veins. These changes are associated with increased renal renin, decreased plasma renin activity, and decreased renal prostaglandin excretion. Increased intrarenal renin synthesis and concentrations of angiotensinogen and angiotensin-converting enzyme (ACE) as a result of low prostaglandin synthesis in the kidney will therefore lead to a vasoconstriction. As a result of renal vasoconstriction by ACE inhibition and angiotensin II inhibitor specific receptors, the occurrence of hemodynamic changes is seen in moderate and severe malnutrition. A study by Alleyne in Jamaica in 1966 proved that in malnourished children there was a decrease in GFR and impaired tubular function [12].

If there is damage to the renal tubules, NGAL levels that excreted in the urine will be high because NGAL can not be properly reabsorbed. Damage to the renal proximal tubules also induces the synthesis and release of NGAL de novo by distal tubular epithelial cells so that NGAL levels in urine increase. Neutrophil gelatinase-associated lipocalin can detect kidney damage, especially renal tubular damage because NGAL is expressed by the tubules and passes through the urine quickly after the kidney is injured and inflamed [13,14,15,16,17].

Urinary NGAL has a sensitivity of 85% and a specificity of 93.33% for renal tubular damage. Research on renal tubular damage in moderate malnutrition children is still very limited. Some studies involved children with severe malnutrition as research subject and some other studies used invasive research methods. Therefore the purpose of this study was to assess the difference in urinary NGAL levels in moderately malnutrition and well-nutrition school-age children in order to be able to detect kidney tubular damage before clinical symptoms manifest.

2. Materials and Methods

This was a cross-sectional study conducted from November until December 2017 on children 6–13 years in Garuda Elementary School in Bandung. Inclusion criterias were children with moderate malnutrition and well-nutrition based on WHO z-score standard reference 2007; not having urinary tract infection according to history taking and physical examination; and received parental consent. Exclusion criteria were children with severely malnutrition, overweight, obesity, had history of suffering from chronic diseases, such as kidney disease heart disease, diabetes mellitus, or malignancy.

Body weight was measured by digital scales (Seca W60094 [1009152], USA) with 10 gram precision. Body height was measured by mobile stadiometer (Seca 217 [CE0123], USA) with a precision of 0.1 cm. Nutritional status was determined using WHOAnthro-Plus software. Data consisted of date of birth, sex, body weight and height were inputted in the appropriate column, then calculated into standard deviation for weight by height (body weight/body height) and body mass index for age (BMI/U) in the form of numbers and decimals. Moderate malnutrition status was defined as body mass index for age <-2 SD and > -3 SD in WHO z-score standard reference 2007. Well-nourished status was defined as body mass index by age below -1 SD to 1 SD or in median range at WHO z-score standard reference 2007. Urine sampling was done by collecting 10 ml of midstream urine in a sterile container, labeled with name, study registry number and date of material collection. The samples were then immediately sent to Hasan Sadikin Hospital laboratory. Urinary NGAL level is examined using ELISA method.

Univariate analysis was used to see the characteristics of subjects including age, gender, urinary NGAL levels, and nutritional status of children in primary schools in Bandung. The data were presented in number and percentage for categorical data such as sex data and nutritional status. Data displayed in the mean, standard deviation, median, minimum and maximum for numerical data such as age and urinary NGAL levels.

Before bivariate analysis, we performed normality test for urinary NGAL level with Shapiro Wilk test for a sample size of less than 50 people and the results were normally distributed when p >0.05. Bivariate analysis were performed to determine the difference between urinary NGAL levels in moderate malnourished children and well-nourished children using Mann Whitney Test when urine levels of NGAL were not normally distributed. Data analysis was performed by *Statistical Product and Service Solution* (SPSS) for Windows version 18.0 on 95% confidence level and p value of 0,05. The research was approved by the Committee of Medical Research Ethics Faculty of Medicine Universitas Padjadjaran Dr. Hasan Sadikin Hospital Bandung.

3. Results

Of the 1,284 populations, 968 children were in well-nourished status, 72 were moderately malnourished, 18 were severely malnourished, 44 were overweight, 3 were obese, 105 were stunted, 4 were severely stunted, and 70 children were unable to be measured. Sample collection was done on 41 children with moderate malnutrition and 40 children with well-nutrition through stratified random sampling. From initial total 81 subjects 1 child with well-nutrition status and 2 children with moderate malnutrition were excluded because they were considered as outlier data. Subjects were divided into moderately malnourished group (n=39) and well-nourished group (n=39). Eighteen children with severe undernutrition, 44 overweight children and 3 children with obesity were referred to Pediatric Nutrition and Metabolic Disease Outpatient Clinic in Hasan Sadikin Hospital. Characteristics of subjects in both groups included the age of the child at the time of the study, sex, weight, height, body mass index, and nutritional status is shown in Table 1.

From the Table 1 shows the differences in age in both groups was not statistically significant with p value = 0.056. Male and female genders in both groups did not differ significantly with p value = 1,000. Differences in body mass index in both groups showed a significant difference with p value <0.01. P value of > 0.05 indicates homogeneity of the subject so that it is considered feasible to be compared. Before the bivariate analysis, we performed normality test of urinary NGAL data with Shapiro Wilk test for sample size less than 50 people and data has normal distribution when p>0.05. Normality test is presented in Table 2.

Normality test showed that age was normally distributed, so the data was presented in the mean and standard deviation. BMI and urinary NGAL variables were not normally distributed, so data was presented in the median and range. Differencial test for urinary NGAL using Mann Whitney test because the data was not normally distributed.

Table 1. Characteristics of Students Population

Characteristics	Nutritional Status		P value
	Well-nourished (n=39)	Moderate Malnourished (n=39)	
Age (years)			
Mean ± SD	11,1 ± 1,1	10,6 ± 1,1	0,056
Range	8,8 – 13,6	8,4 – 12,7	
Sex, n (%)			
Male	27 (69,2)	27 (69,2)	1,000
Female	12 (30,8)	12 (30,8)	
BMI (kg/m^2)			
Median	15.7	13.6	<0,01
Range	14,2 – 22,4	12,9 – 14,5	

Note: SD = Standard Deviation, n=frequency, %=percentage, BMI = Body Mass Index.

Table 2. Normality Test of Data Distribution

Variables	Nutritional Status	Shapiro-Wilk	
		P value	Distribution
Age (years)	Well-nourished	0.124	Normal
	Moderate malnourished	0.151	Normal
BMI (kg/m2)	Well-nourished	0.000	Abormal
	Moderate malnourished	0.016	Abormal
Urinary NGAL	Well-nourished	0.000	Abormal
	Moderate malnourished	0.000	Abormal

a. Lilliefors Significance Correction.

Table 3. The Difference of Urinary NGAL levels in Moderate malnourished and Well-nourished

Variables	Nutritional Status		Differences (95% CI)	P value*
	Well-nourished (n=39)	**Moderate malnourished** (n=39)		
Urinary NGAL Level				
Median	12,72	56,27	43,6	<0,001
Range	0,31–45,61	41,91–114,23	38,3 – 50,1	

*)Mann Whitney Test.

From the results of the analysis in Table 3, there were significant differences in urinary NGAL levels between the children with well-nourished and moderate malnutrition (p <0.05). Median urinary NGAL levels in the group of children with moderate malnutrition were higher than those of well nutritional status with a difference of 43.6 with 95% CI between 38.3–50.1. Data distribution of urinary NGAL of moderate malnourished and well-nourished children is seen in Figure 1.

Figure 1 shows the data distribution of urinary NGAL level between well-nourished group and moderate malnourished group. It is seen that data distribution of moderate malnourished group is higher than well-nourished group.

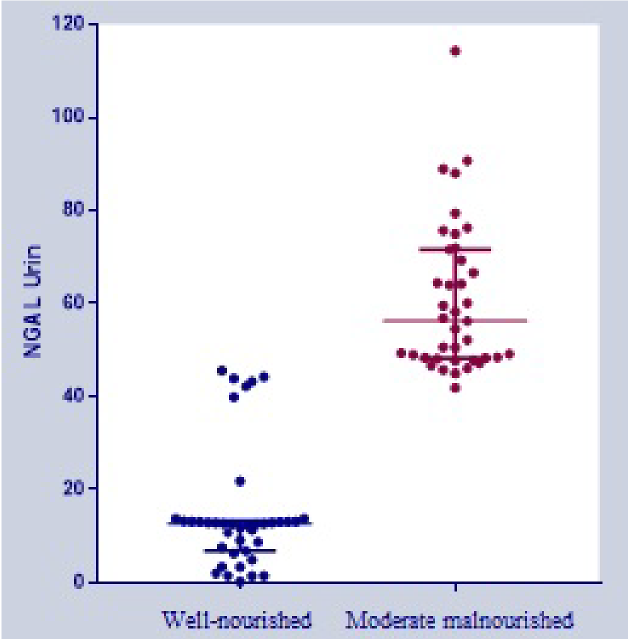

Figure 1. Scatter plot the difference of urinary NGAL levels in well-nourished and moderate malnourished children

4. Discussions

Examination of serum and urinary NGAL levels is particularly useful in patients with acute renal failure. In some studies, NGAL levels in the urine may increase within 2 hours after kidney damage and increase within 4–6 hours in blood. To determine the presence of kidney damage, urinary NGAL is preferred than serum NGAL due to its specificity and rapidity because elevated serum NGAL levels are also influenced by many other factors such as inflammatory conditions. In renal disease especially when tubular damage occurs, urinary NGAL levels will be highly increased and positively correlated with the severity of kidney damage occurring [14,18,19,20,21,22].

Neutrophil gelatinase-associated lipocalin is a small lipocalin group protein (21 kDa), present in neutrophils and other epithelial cells, especially in renal tubular cells. Normal serum NGAL values in humans are 30.51-105.80 µg/L and are not differentiated by age and gender [21]. Some studies suggested a renal impairment when the cut-off value of urinary NGAL in children is 100–135 ng / mL [23]. These levels of protein in blood and urine will increase to 1000 times from normal levels within 2-6 hours after kidney injury. This study involved 39 children with moderately malnutrition and 39 children with well nutritional status. Parameters used in this study was urinary NGAL level. Our results showed that age and gender in both groups did not significantly differ statistically and had no association with elevated urinary NGAL levels.

A research conducted by Klahr and Tripathy in 1966 in adult patients with malnutrition found a reduction in creatinine weight and inulin clearance. In this study the rate of glomerular filtration was decreased, serum creatinine was normal, and blood urea nitrogen (BUN) levels were low. As the protein level started to recover, the glomerular filtration rate was back to normal or near normal, and serum creatinine, BUN and albumin all returned to normal. This showed an increase in protein synthesis and muscle mass [24]. In this study we did not use glomerular filtration rate, serum creatinine, and BUN levels as parameters because the methods required would be invasive and nevertheless did not describe kidney tubular damage.

Alleyne's study (1967) used GFR and inulin clearance parameters in moderate malnourished children found that there was a decrease in GFR and aminoaciduria in undernourished children but no significant correlation between inulin clearance and weight loss. The presence of a decrease in GFR and aminoaciduria in undernourished children proves the presence of glomerular function disorders and renal tubules. Some evidence of renal tubular disorders were the presence of magnesium and potassium deficiency. [12] Research on kidney function in moderate malnourished and well-nourished children with creatinine and cystatin C serum as parameter had been done in Hasan Sadikin General Hospital in 2012. From this study we found that creatinine serum was lower and cystatin C was higher in moderately malnourished children than well-nourished children [25].

We used the urinary NGAL level examination in this study because if there is damage to the renal tubules, NGAL levels that excreted in the urine will be high because NGAL can not be reabsorbed properly. Damage to the renal proximal tubules also induces the synthesis and release of NGAL de novo by distal tubular epithelial cells so that NGAL levels in the urine increase. Neutrophil gelatinase-associated lipocalin can detect kidney damage, especially renal tubular damage because NGAL is expressed by the tubules and passes through the urine quickly after the kidney is injured and inflamed [13,14,15,16,17].

Other studies that used NGAL as a biomarker include research by Yazdani and colleagues using serum NGAL levels as an iron deficiency anemia biomarker in children with chronic kidney disease. In that study, it was found that serum levels of NGAL were increased in children with low transferrin saturation levels [26]. The study by Rajit and colleagues comparing urinary NGAL levels with serum creatinine as a predictor of renal tubular damage in patients with acute post-renal failure heart surgery found that urinary NGAL was the best biomarker as a predictor of renal tubular damage and acute renal failure compared to serum creatinine levels [27].

It was concluded in this study that the median of urinary NGAL level in children with moderate malnutrition is higher than in children with well-nourished with difference of 43,6 (95% CI between 38,3-50,1). Undernutrition affects renal function in the decrease of glomerular filtration rate (GFR), sodium and phosphate excretion, lower erythropoietin synthesis. It is related to kidney mass of moderate malnourished children that is less than well-nourished children [9,10,28,29]. Increase in urinary NGAL levels in malnourished children is a sign of tubular damage and is expected to return to normal if the nutritional status recovers. Damage to the renal tubules if not intervened will lead to a high risk of acute renal impairment.

In this study we found that 6 out of 39 well-nourished children who experienced elevated levels of urinary NGAL. NGAL levels in the urine will be greatly increased and positively correlated with the severity of kidney damage occurring [21]. NGAL is expressed by the tubules and excretes in the urine shortly after the kidney is injured and inflamed [13,14,15,16,17]. Hence, the presence of infection such as urinary tract infection might be the reason of the elevated levels of urinary NGAL in 6 children with good nutritional status. Further examination to determine the etiology of urinary NGAL increase in children with good nutrition is needed.

Limitations of this study were cross sectional method that only assessed nutritional status and urinary NGAL levels at one time. Another limitation was that no examination was done to exclude asymptomatic urinary tract infections and actually more data was required on risk factors for renal tubular damage including birth weight history or congenital renal abnormalities. Follow-up examinations to determine the adverse effects of renal tubular damage such as magnesium and potassium deficiency also were not performed and the investigators did not do follow up examination on the levels of urinary NGAL in undernourished children who received nutritional interventions.

5. Conclusion

There is a significant difference in urinary NGAL levels of moderate malnutrition and well-nourished children so that there is a possibility of renal tubular damage in moderate malnutrition children even though no symptoms are manifested. Group of children with poor nutritional status need optimal management and increased awareness to prevent long-term effects of renal and cardiovascular complications. Education needs to be done about healthy eating and prevention of chronic infections, especially in the parents of school-aged children to break the chain of moderate and severe undernutrition which have a high level of morbidity and mortality. Further research is needed on the impact of renal tubular damage that occurs in children with poor nutritional status. Subsequent research can use a cohort method to monitor growth and development despite the renal impairment that has occurred. Further studies are also required on urinary NGAL levels in children with undernutrition who have received nutritional interventions.

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Conflict of Interest

There is no conflict of interest in conducting and publishing this research.

Disclaimers

This study has not been published previously, not under consideration for publication elsewhere, and its publication is approved by all authors. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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