

Correlation between Neutrophil-to-Lymphocyte Ratio (NLR) and Immature-to-total Neutrophil (I/T) Ratio to Bacterial Infection among Children with Chronic Kidney Disease

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Abstract

Background: Chronic kidney disease (CKD) in children has a long-lived impact, such as an increased risk of bacterial infection. Infection may accelerate disease progression, making early detection crucial. Inflammatory markers typically used for bacterial infection are C-Reactive Protein (CRP) and Procalcitonin (PCT). This study aimed to determine the correlation between levels of neutrophil-to-lymphocyte ratio (NLR) and immature-to-total-neutrophil ratio (I/T ratio) to bacterial infection in children with CKD as indicated by the serum levels of CRP and PCT.

Methods: Observational analysis with a cross-sectional design was conducted from January 2019 to November 2021 in children from 3 months to 18 years old with CKD and bacterial infection. Retrospective data were obtained from medical records at Dr. Hasan Sadikin General Hospital, Bandung. Correlation analysis was performed (SPSS program) at a 95% confidence level, and results were considered significant if the p-value <0.05.

Results: There were 42 children, and 57% were female; with a median age of 13 years (range 1-17 years). Most patients had normal nutritional status (55%) although 40% were malnourished. Correlation analysis between I/T ratio and NLR with PCT was positive, with $r=0.284$ ($p<0.05$) and $r=0.265$ ($p<0.05$), respectively, whereas there was no significant correlation of I/T ratio ($r=0.154$; $p>0.05$) and NLR ($r=0.188$; $p>0.05$) to CRP.

Conclusions: NLR and I/T ratios have a significant positive correlation with PCT levels but not with CRP levels. NLR and I/T ratios can be considered as alternative markers for diagnosing CKD in children with a bacterial infection.

Keywords: Bacterial infection, c-reactive protein, children, chronic kidney disease, procalcitonin

Introduction

Chronic Kidney Disease (CKD) is a kidney dysfunction affecting children and adults that chronically progressed.^{1,2} This definition has been coined by Kidney Disease Improving Global Outcomes (KDIGO), which was updated in 2012.² The incidence and prevalence of CKD have increased by 90% from 1990 to 2016,³ and varies in many countries. A multicentre study in Turkey has reported that the CKD incidence is 10.9 cases per 1,000,000 children, in which 68% of cases progressed to End Stage Renal Disease (ESRD) with leading cause of death is cardiovascular diseases followed

by infection. The bacterial infection is also strongly suspected as the major complication of CKD in Indonesia.⁴

The primary etiology of CKD in children is congenital anomalies of the kidney and urinary tract (CAKUT), followed by steroid-resistant nephrotic syndrome (SRNS), chronic glomerulonephritis, and renal ciliopathy.⁵ Therefore, the clinical manifestation of CKD in children is varied. Depending on the etiology, CKD could manifest into hematuria, edema, polydipsia, polyuria, nocturia, and enuresis resulting in poor linear growth and hypertension. The KDIGO classifies CKD based on the etiology, glomerular filtration

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rate (GFR) categories, and albuminuria. Furthermore, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) has grouped CKD based on the GFR, that is divided into five stages categorizes i.e. stages 1–3 as early stages and 4–5 as later stages, whereas stage 5 is the end stage of renal disease (ESRD).⁶

Decreased renal clearance in CKD leads to immune dysfunction and an increased inflammation activity.^{7,8} Children with CKD are also prone to infection, and the most common infections are urinary tract infection (UTI), pneumonia, and sepsis.¹ These conditions may lead to systemic inflammatory response syndrome, resulting in poor prognosis, increased disease burden, and accelerated CKD progression; hence an early diagnosis of infection is crucial.^{1,9} Culture has been recognized as the gold standard for definitive diagnosis of bacterial infection. However, it is rarely used due to its cost and resource limitations. Some examinations that can give a clinical picture of bacterial infection are among other white blood cell (WBC), platelet count, erythrocyte sedimentation rate (ESR), absolute neutrophil count (ANC), c-reactive protein (CRP) and procalcitonin (PCT). The CRP and PCT have an acceptable specificity for diagnosing bacterial infection in a patient with kidney dysfunction.^{10,11} Other parameters used to detect bacterial infection are the neutrophil lymphocyte ratio (NLR) which is increased acutely after bacterial infection (<6 hours), and the immature/total neutrophil ratio (I/T ratio).^{12,13} Quick response of NLR is beneficial not only for the early assessment of bacterial infection but also helps diagnose sepsis, pneumonia, and COVID-19.^{14–17} The progressive decrease in kidney function will result in an increase in polymorphonuclear cells, which in turn increases the I/T ratio.¹⁸ The NLR and I/T ratio examinations are inexpensive and easily found in primary health care with limited resources. In this study, we analyzed PCT and CRP as typical indicators of bacterial infection. This study aimed to prove a positive correlation between NLR and I/T ratio on the incidence of bacterial infection in children with CKD, as indicated by the CRP and PCT serum levels.

Methods

A cross-sectional analytic observational study was conducted using retrospective data from patients' medical records. Data were collected from the central electronic database (*Sistem*

Informasi Rumah Sakit, SIRS) in Dr. Hasan Sadikin General Hospital, Bandung, between January 2019 and November 2021. Inclusion criteria were children aged three months to 18 years old who diagnosed with CKD stage 1–5 and had been diagnosed bacterial infection and had received antibiotic treatment. Data on complete blood count, including differential count, NLR, I/T ratio, CRP, and PCT were collected from the Laboratory. Exclusion criteria were children with diabetes mellitus, a hematopoietic system disorder, a history of malignancy and chemotherapy, an acute CKD or otherwise with incomplete data.

The sample size was calculated with a significance level of 5%, a 90% power test, and a correlation coefficient of 0.377, resulting in a minimum sample size of 42 subjects. Characteristics of patients were shown in the frequency table. Categorical variables were described by numbers and proportions, while numerical variables were described in terms of mean, standard deviation (SD) or median, and range depended on the data distribution. A correlation analysis was performed with the Pearson correlation test if the variables were normally distributed or Spearman's rank if not normally distributed. Variance analysis or the Kruskal-Wallis test was used to compare the differences between variables. Multivariate analysis was conducted on possible confounding variables (age, gender, and nutritional status) using linear regression. Variables were included in the analysis if the result was significant with a 95% confidence interval. The results were considered significant if the p-value ≤ 0.05 . The data analysis had used SPSS 20.0. This study obtained permission and approval from the Ethics Committee of Dr. Hasan Sadikin General Hospital with number LB.02.01/X.6.5/371/2021.

Results

Out of 126 patients, 84 were excluded due to incomplete data and a history of chemotherapy, remaining a minimum data (n=42) of children with CKD. The characteristics of the children had shown that more than half were female (n=24; 57%) with a median age of 13 years (range 1–17 years) and most of the children were between 10 to 15 years old (67%) (Table 1). The children had normal nutritional status (n=23; 55%), however, 40% children were malnourished, and 5% patients were obese.

The laboratory parameter (Ratio I/T, NLR, CRP, dan PCT) were not normally distributed (Shapiro-Wilk test) as shown in Table 2. The

Table 1 Characteristics of Children with Chronic Kidney Disease (n=42)

Characteristic	Frequency (n)	Percentage (%)
Gender		
Male	18	43
Female	24	57
Age (year)		
1-9	6	14
10-15	28	67
16-18	8	19
Median: 13		
Range: 1-17		
Nutritional status		
Severe malnutrition	6	14
Moderate malnutrition	11	26
Normal	23	55
Obesity	2	5

Table 2 Laboratory Results in Children with Chronic Kidney Disease and Normality Test

Variable	Statistical value			Normality test (p-value) *
	Mean (SD)	Median	Range	
Ratio I/T	0.0 (0.014)	0.0	0.0-0.075	<0.001
NLR	7.30 (5.97)	5.52	0.75-28.29	<0.001
CRP	5.81 (5.16)	4.57	0.22-23.27	<0.001
PCT	52.60 (94.14)	17.56	0.08-509.72	<0.001

Note: *Based on Shapiro-Wilk test, I/T= immature/total neutrophil, NLR= Neutrophil lymphocyte ratio, CRP= c-reactive protein, PCT= procalcitonin

correlation between I/T ratio as well as NLR with PCT showed that there was a positive correlation with $r=0.284$ ($p=0.034$) and $r=0.265$ ($p=0.045$), respectively (Table 3). NLR increased as the value of the I/T ratio increased. In contrary, there was no correlation between I/T ratio or NLR with CRP with $r=0.154$ ($p=0.165$) and $r=0.188$ ($p=0.116$), respectively (Figure 1).

Discussion

Our study has explored NLR and I/T ratio in relation to infection marker CRP and PCT in children with chronic kidney disease (CKD). The majority of the children in this study were female (57%), in contrary to other study showing that male is more prevalence.¹⁸ Interestingly, the frequency of congenital

Table 3 Correlation of I/T Ratio or NLR with CRP and PCT in Children with Chronic Kidney Disease

Correlation	Correlation coefficient (r)	p-value*
I/T ratio with CRP	0.154	0.165
I/T ratio with PCT	0.284**	0.034**
NLR with CRP	0.188	0.116
NLR with PCT	0.265**	0.045**

Note: *) One-tailed test, **) Significant, I/T= immature/total neutrophil, NLR= Neutrophil lymphocyte ratio, CRP= c-reactive protein, PCT= procalcitonin

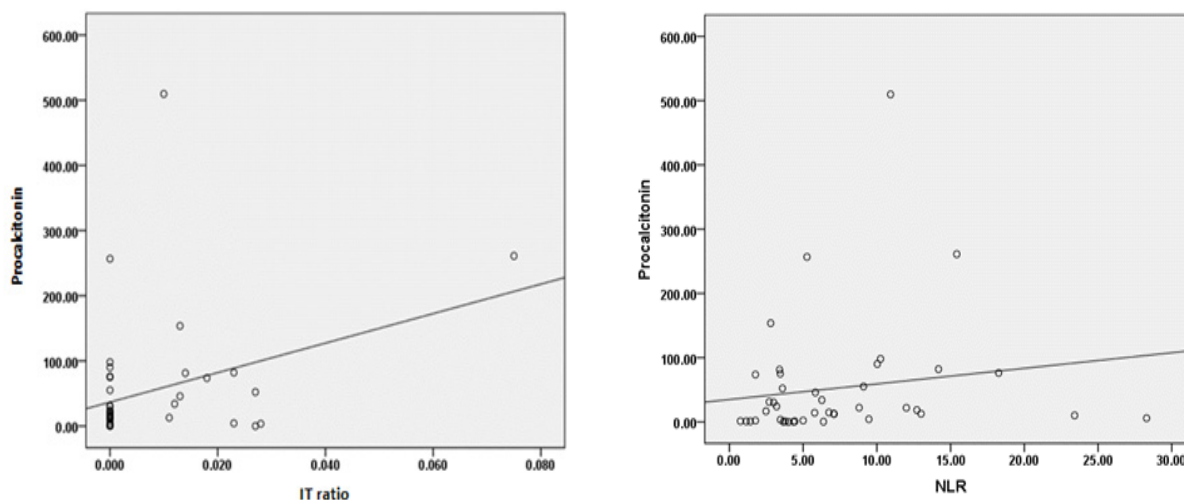


Figure Correlation between I/T Ratio (a) or NLR (b) and PCT in Children with Chronic Kidney Disease

kidney disease known as Congenital Anomalies of Kidney and Urinary Tract (CAKUT) is higher in males.¹ However, this study did not highlight CAKUT as the primary etiology of CKD. Additionally, this study had a smaller number of subjects. The age of children with CKD in this study ranged from 1 to 17 years old, dominated by the 10–15 age group with a median of 13 years old. Several other studies have shown various median age of 10 years old,¹⁸ and others with younger age of 6 or 8 years old.¹⁹

Low-grade chronic inflammations play a significant role in CKD progression and complication.⁸ Kidney injury, hypoalbuminemia, decrease in cytokine and metabolite clearance may result in inflammation and proteinuria significantly and contribute to further kidney injury and pro-inflammation condition with increasing cytokine production.²⁰ Uremic environments in CKD produce oxidative and carbonyl stress that are highly pro-inflammatory.⁸ Levels of inflammation biomarkers in CKD patients, such as CRP, interleukin (IL)-6, and TNF- α are elevated.²¹ Increased inflammatory proteins are strong predictors of mortality, including cardiovascular deaths in patients with ESRD.^{7,8}

This study analyzed the correlation of the I/T ratio and NLR with frequently used inflammatory markers CRP and PCT in patients with CKD. The I/T ratio obtained in this study has a median 0% (range 0–0.075%). For sepsis diagnosis, the the I/T ratio cut-off value of >0.2% has a high sensitivity and specificity.²² However, previous studies never

mentioned the value of I/T ratio in children with CKD. A study of CKD in adults has shown that I/T ratio of 68.7% is significantly higher than patients without infection.²³ In this study, NLR has a median of 5.52 (range 0.75–28.29), in contrary to other study that shows a lower result (mean 2.48 ± 2.96).²⁴ Interestingly, there is a significant difference between general population (1.75), patients with CKD not requiring dialysis (2.54), patients with CKD who required dialysis (2.42), and patients with CKD using peritoneal dialysis (3.15).²⁵

Relationships between inflammatory markers in children with CKD have not been studied. With $r=0.265$ (p -value=0.045), we inferred a low positive correlation between NLR and PCT, according to Guilford criteria. However, positive correlation is found between NLR and PCT in adult patients with ESRD, with $r=0.285$ (p -value <0.001).²⁶ Moreover, PCT <0.5 and ≥ 0.5 have been associated with NLR.²⁷ Neutrophil extracellular traps (NET) are responsible for the role of neutrophil in the pathology of CKD and the increase of NLR. NET is a defense mechanism mediated by neutrophils, trapping bacterial pathogens by releasing DNA out of the cell compartment.²⁸ In a patient with uremia, NET is associated with NLR and neutrophil count.^{28,29} This finding suggests that NET is involved in the elevation of NLR, suggesting a possibility of having NLR as a new therapeutic target in CKD. A positive correlation is also found between the I/T ratio and the level of PCT. A cut-off >0.2% for inflammatory disease has been set.²² Interestingly, other studies revealed

neutrophil count that is relatively lower in neonates compared to adults. The maximum I/T ratio in neonates without infection is 0.16% in the first 24 hours and decreases into 1.2% within 60 hours after birth. Age is also associated with the decrease of lymphocyte; a drastic decrease occurs between birth, young adulthood, and above 40 years old.³⁰ In this study, age, gender, and nutritional status, which were considered confounding factors, were not proven.

This study has some limitations. We cannot obtain serial exams on the study variables that would provide insight into the variable's trend. Moreover, in some patients, the timing of sample collection might not be at the time of disease onset. Although no correlation was found between the I/T ratio and NLR with CRP, however, the I/T Ratio and NLR tended to have a positive correlation with CRP. One reason might be a differences in sample size. A power test with a greater sample size might be needed to overcome this bias. However, this is the first study analyzing the correlation between NLR and the I/T ratio to the incidence of bacterial infection in children with CKD.

In conclusion, the I/T ratio and NLR are considered suitable alternatives for bacterial infection marker such as PCT in patients with CKD. This will help early diagnosis in health care facilities with limited resources to treat bacterial infection in children with CKD. A further longitudinal study with a larger sample size using serial examination is needed to confirm the correlation between the I/T ratio and NLR with CRP and decide on the cut-off for each variable.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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