



CORRELATION OF THE NEUTROPHIL-TO-LYMPHOCYTES RATIO WITH SERUM UREA AND CREATININE LEVELS IN LEPTOSPIROSIS PATIENTS

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ABSTRACT

Background: Leptospirosis is a zoonosis that is still a problem in Indonesia. The kidneys are often the target organs in leptospirosis. The outer membrane of *Leptospira* sp. contains antigenic components and toxins that stimulate the inflammatory process in the kidneys. The neutrophil-to-lymphocyte ratio (NLR) is used as a marker of the inflammatory process, while urea creatinine is a sign of kidney damage. **Objective:** To prove the correlation between NLR and serum urea and creatinine levels in leptospirosis patients. **Methods:** This study was carried out retrospectively on the medical records of patients with confirmed leptospirosis by Microscopic Agglutination Test (MAT) at Dr. Kariadi Semarang between January 2020 and July 2022. The NLR was calculated by dividing the number of neutrophils by the number of lymphocytes in the leukocyte count, urea, and creatinine levels from the patient's medical record. Statistical analysis using the Spearman correlation test with a significance level of $p < 0.05$. **Results:** From 33 samples, the median NLR was 10.75 (2.19-47.5), the median urea level was 131.0 mg/dL (15-467 mg/dL), and creatinine was 2.0 mg/dL (0.7-11.9 mg/dL). There is a correlation between NLR and urea ($p = 0.001$, $r = 0.561$) and creatinine ($p = 0.003$, $r = 0.501$). An increase of NLR, urea, and creatinine were found in leptospirosis patients. **Conclusion:** There is a moderate positive correlation between NLR and urea and creatinine. An increase in NLR is a sign of an inflammatory process from leptospirosis, which correlates with an increase in urea and creatinine.

Keywords: Neutrophil-to-lymphocyte ratio, Urea, Creatinine, Leptospirosis

BACKGROUND

Leptospirosis is an acute infectious disease that affects humans and animals. This disease is a zoonotic disease caused by the species *Leptospira interrogans* and the *spirochaete* group.¹ Leptospirosis occurs worldwide but is most common in countries with tropical and subtropical climates that experience heavy rains, one of which is Indonesia.²⁻⁴ Leptospirosis is still a threat to public health in Indonesia because of the presence of risk factors, namely the high population of rats (rodents) as a leptospirosis reservoir, poor environmental sanitation, and the increasingly widespread flood areas in Indonesia. Until now, leptospirosis in Indonesia has continued to spread and cause human death.⁵

Based on data from the Central Java Provincial Health Office, in 2016, there were 164 cases of leptospirosis, of which 30 died. This number increased in 2017, namely, there were 409 cases of leptospirosis, and 65 of them died. In 2018, there were 427 cases, and 89 of them died. In 2019, the number of leptospirosis cases increased by 458, and 67 of them died. In 2020, the number of leptospirosis cases was still high, with 422 cases, of which 49 died.

In the city of Semarang in 2021, there were 23 cases of leptospirosis, and five of them died. From the above data, it can be seen that the incidence of leptospirosis and mortality in Central Java is high, and Semarang is one of the cities with the highest incidence of leptospirosis in Central Java.⁶

Leptospire that have entered the body are widely distributed hematogenously, and some of them enter the proximal kidney tubule cells. The kidney is one of the target organs in leptospirosis, and interstitial tubular nephritis is the most common clinical and pathological manifestation.^{1,7} The outer membrane of *Leptospire* contains antigens and toxins that act as inflammatory mediators in the kidney.⁷⁻⁹

The neutrophil-to-lymphocyte ratio (NLR) is a reliable and easy to get marker of the immune response against a wide range of infectious and non-infectious conditions. The normal range of NLR is between 1-2; values higher than 3.0 and below 0.7 in adults are considered pathological. NLR in the gray zone between 2.3-3.0 can serve as an early warning of pathological conditions or processes such as cancer, atherosclerosis, infection, inflammation, psychiatric disorders, and stress. NLR is a very



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sensitive indicator of infection, inflammation, and sepsis. Clinical studies confirm the sensitivity of NLR for the diagnosis of systemic infection, sepsis, and bacteremia and its strong predictive and prognostic value. The severity of critical illness and serious inflammation is expressed by an increase in the NLR value above 11. Improved clinical course of sepsis, critical illness, and lower risk of death are associated with a decrease in NLR values below 7.¹⁰

In leptospirosis, leukocytes, especially neutrophils and lymphocytes, are one of the signs of an inflammatory process.^{11,12} According to a study by De Silva et al., the number of neutrophils tends to increase in severe leptospirosis, and the number of lymphocytes tends to decrease in severe leptospirosis, resulting in an increased NLR rate in leptospirosis.^{13,14}

In previous case studies, it was found that urea and creatinine levels increased in cases of severe leptospirosis. Urea and creatinine levels will change along with changes in the patient's kidney condition.¹⁴⁻¹⁷ According to a study by Nisansala et al., Acute kidney injury (AKI) is a complication that often occurs in patients with confirmed leptospirosis, characterized by an increase in creatinine serum.¹⁸

This study aims to analyze the correlation between the neutrophil-to-lymphocyte ratio (NLR) and urea creatinine serum levels in leptospirosis cases. This research was conducted due to the limited research on these parameters in leptospirosis patients.

METHODS

This research was conducted using a retrospective cross-sectional method on the patient's medical record, which confirmed leptospirosis by microscopic agglutination test (MAT) at RSUP Dr. Kariadi Semarang from January 2020 to July 2022 with a titer $\geq 1/160$ ^{15,19}. Subjects who met the inclusion criteria were not pregnant women, had no history of kidney disease or malignancy, and were not currently receiving kidney-related therapy.

There were 33 research subjects who then recorded the value of the neutrophil-to-lymphocyte ratio obtained by dividing the absolute neutrophil count by absolute lymphocyte count using an automated hematology analyzer, the Sysmex Xn-1000. Urea and creatinine serum levels were

examined using the ADVIA 1800 chemistry system. The NLR reference value in 95% of healthy adult patients is between 0.78 and 3.53; the serum urea reference value is 6-20 mg/dL; and the serum creatinine reference value is 0.6-1.3 mg/dL.

Descriptive analysis displays the median, minimum, and maximum values of the variables. The data normality test was performed with Shapiro-Wilk. The Spearman's correlation test was performed to determine the correlation between NLR and urea and creatinine levels with level significance $p < 0.05$. This research has received ethical approval from the Ethics Committee of RSUP Dr. Kariadi Semarang, with the number DP.02.01/I.II/6096/2022.

RESULTS

A total of 33 subjects with leptospirosis who met the inclusion and exclusion criteria consisted of 20 males and 13 females. The distribution of subject characteristics is presented in Table 1.

Table 1. Characteristics of research subject data

Variable (n= 33)	Median (min-max)
Age (years)	47 (16-81)
Haemoglobin /Hb (gr/dL)	12.20 (7.30-15.60)
Leukocytes count ($\times 10^3 / \mu\text{L}$)	14.70 (2.50-34.90)
Platelets count ($\times 10^3 / \mu\text{L}$)	143.00 (18.00-370.00)
Neutrophils count (%)	81 (48-95)
Lymphocytes count (%)	8 (1-29)
Neutrophil-to-lymphocyte ratio / NLR	10.75 (2.19-47.5)
Urea (mg/dL)	131(15.0-467.0)
Creatinine (mg/dL)	2.6 (0.7-11.9)

Min (minimum); Max (maximum); Data distribution is not normal ($p < 0.05$), normality test with Shapiro Wilk.

Laboratory data analyzed included neutrophil count, lymphocyte count, NLR, serum urea, and creatinine levels. The correlation between NLR and serum urea and creatinine levels using the Spearman Rank test can be seen in Table 2.

Table 2. Correlation of neutrophil-to-lymphocyte ratio with serum urea and creatinine levels

Variable	Neutrophil-to-lymphocyte ratio (NLR)	
	<i>p</i>	<i>r</i>
Urea (mg/dL)	0.001*	0.561
Creatinine (mg/dL)	0.003*	0.501

* $p < 0.05$; *r*, coefficient correlation, correlation test with Spearman Rank

The results of the data analysis showed that there was a moderate positive correlation between the ratio of neutrophils-to-lymphocytes ratio and levels



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of serum urea ($p = 0.001$; and $r = 0.561$) and serum creatinine ($p = 0.003$; and $r = 0.501$).

DISCUSSION

Leptospira enter the bloodstream through *port d'entrée* in the skin, oral mucus membranes, nose, conjunctiva, and so on, which then multiply and spread through the bloodstream to organs and body tissues. Leptospirosis is characterized by the presence of vasculitis, endothelial damage, and inflammation consisting of monocytes, plasma cells, histiocytes, and neutrophils. On physical examination, bleeding and jaundice are common.¹

Histopathology is most prominent in the liver, kidney, heart, and lungs, but other organs may also be affected according to the severity of the infection. The structure of the liver is not significantly disturbed, but there may be intrahepatic cholestasis.

In the kidney, interstitial nephritis is the main finding, accompanied by leukocyte infiltration consisting of neutrophils and monocytes. *Leptospira* can be seen in the kidney tubules with the assistance of an electron microscope, the tubular cell boundaries appear thin, the tubular basement membranes are thickened, and tubular cells show mitochondrial thinning. In addition, small changes are seen in the glomerulus, suggesting the anatomical basis of proteinuria in leptospirosis.¹

Leptospira attaches to renal epithelium cells, and its adhesion is enhanced by agglutination antibodies. The outer membrane of *Leptospira* consists of lipopolysaccharide (LPS) and several lipoproteins which are immunogenic, one of which is lipopolysaccharide type 32 (LipL32). *Leptospira* LPS stimulates neutrophil attachment to endothelium cells and platelet aggregation, and it is thought to play a suspected role in the occurrence of thrombocytopenia.¹ LipL32 is related to innate immunity because it directly induces an inflammatory response. Most often, this leads to tubulointerstitial nephritis as large amounts of inflammatory mediators, including the chemoattractant monocyte protein-1 (MCP-1), inducible nitric oxide synthase (iNOS), and tumor necrosis factor- α (TNF- α) are produced in the surrounding kidney cells. In addition, Areán et al., proved that LipL32 also acts as a hemolysin, which in turn induces proinflammatory cytokines through various toll-like receptor (TLR) signaling pathways.^{1,8}

Inflammation that occurs in leptospirosis causes an increase in the number of leukocytes and neutrophils according to the severity of the disease.^{11,20} In this study, there was an increase in the percentage of neutrophils with a median of 81%, lymphocytes with a median of 8%, and NLR with a median of 10.75. The results of this study are in line with the research of Nisansala et al., which found an increase in the percentage of neutrophils, a decrease in the percentage of lymphocytes, and an increase in serum urea creatinine level. The study also mentions that fever is the most common clinical symptom and that AKI is the most frequent complication of leptospirosis.^{18,21} Urea levels with a median of 131.0 mg/dL and creatinine levels with a median of 2.0 indicate kidney disorders due to an inflammatory process. The results of this study are in line with the research of Nisansala et al., that the most complications are in the kidneys. This study also showed that there was a correlation between high levels of NLR and high levels of urea and creatinine.¹⁸ High levels of NLR can predict kidney disorders.^{20,22}

This study does not distinguish between the degree of disease severity and the type of serovar that causes that infection. Further research is needed to compare the severity of the diseases and the types of *Leptospira* serovars that cause infection on NLR parameters and kidney functions in leptospirosis patients.

CONCLUSION

This study gave the results of a moderately positive correlation between NLR and serum urea and serum creatinine levels. A higher NLR value can be associated with kidney disorders.

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