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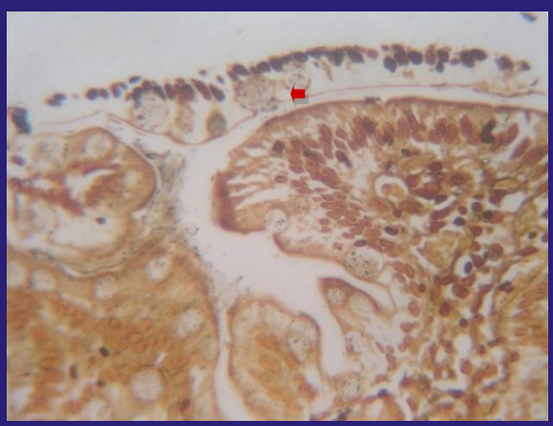

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CONTENTS

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Original Article

Neutrophil-to-lymphocyte and Platelet-to-lymphocyte Ratio as Predictors of CD4 Count among People Living with HIV

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ABSTRACT

Human Immunodeficiency Virus (HIV) infection remains a global health concern characterized by the reduction of CD4 lymphocyte cells and weakened immune systems. Knowing the CD4 count and the factors affecting it is crucial for assessing the immune status of HIV patients. Hematological markers, including neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), have been recognized as prognostic tools that were associated with CD4 Count. The goal of this study was to determine the impact of NLR and PLR on CD4 count among people living with HIV (PLHIV). This study used analytic observational method with a cross-sectional on HIV-positive individuals who were treated at Moewardi Hospital, Surakarta, Indonesia. The Chi-Square and Pearson correlation tests were performed to identify the correlation between variables and the linear regression test was done to investigate the association between NLR and PLR with CD4 count. A total of 80 PLHIV were identified for this study, with the median CD4 count of 103 cells/mm3. NLR and PLR were found to be 3.06 and 181.03, respectively. This study found that opportunistic infection, duration on ARV treatment, body mass index, total lymphocyte count, and hemoglobin were significantly associated with CD4 count. The Pearson correlation test revealed a strong correlation between NLR and PLR to CD4 count. Linear regression analyses showed that NLR and PLR could predict the CD4 count. These findings indicate that NLR and PLR could serve as alternative prognostic parameters for monitoring treatment outcomes in PLHIV, particularly in health facilities where access to CD4 count testing is limited.

Keywords: HIV, Neutrophil to Lymphocyte Ratio, Platelet to Lymphocyte Ratio, CD4 count, and prognostic factor.

Highlights: This study confirms the predictive role of NLR and PLR in CD4 count as the indicator of immune status in PLHIV. Both of these are widely available markers that can aid clinicians in monitoring HIV patients' immune status, thereby reducing morbidity and mortality from HIV infection.

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INTRODUCTION

Human Immunodeficiency Virus (HIV) infection has become a health challenge with increasing case rates worldwide. According to UNAIDS data, a total of 39 million people worldwide had HIV and approximately 1.3 million of them were newly diagnosed in 2022.[1] Since the first cases were officially reported in 1981, a total of 84.2 million cases of HIV have been found.[2] The primary targets of HIV infection are the Cluster of Differentiation 4 (CD4) and chemokine receptors, both of which can be found in several human immune system cells.[3] These cells include helper T lymphocytes, dendritic cells, and macrophages.[3] Viruses attached to receptors on target cells will proceed with membrane fusion, allowing the viral components to enter the cells and replicate.[4] Continued replication will destroy target cells, decreasing CD4 levels.[5] This leads to impaired immunity and susceptibility to various opportunistic infections.[6]

Examining CD4 counts needs to be done regularly to determine the immune status of HIV patients.[6] However, there are several obstacles in conducting the examination, such as high examination fees and limited availability of this service in health facilities.[7] For this matter, it is crucial to discover alternatives to CD4 tests to monitor the immune status of HIV patients. Over the past few years, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have emerged as systemic inflammatory indicators and predictive factors of death in the general population.[8] NLR reflects both types of the body's immune response, neutrophils as an innate response and lymphocytes as an adaptive response.[9] Meanwhile, PLR simultaneously connects inflammatory pathways and aggregation.[10]

HIV infection is linked with immune hyperactivation and chronic inflammation, characterized by rising levels of various proinflammatory cytokines and hypercoagulable biomarkers.[11] High NLR and PLR have been shown to reflect severity and were linked with increased mortality in PLHIV as a result of the immunosuppression linked to low CD4 count.[9] One of the challenges in the clinical management of PLHIV is the limited access to CD4 count service in primary healthcare facilities while there is hematologic testing in these facilities. Despite the potential role of NLR and PLR to predict CD4 count, there is limited evidence in this area. Therefore, the goal of this study was to determine the correlation between NLR and PLR on CD4 count among PLHIV.

MATERIALS AND METHODS

This research is characterized as a cross-sectional study carried out at the Voluntary Counselling and Testing Polyclinic of Moewardi Hospital, Surakarta, Central Java, Indonesia, involving patients with HIV from 2017 – 2023. The participant inclusion criteria included patients diagnosed with HIV aged 18 - 65. The exclusion criteria encompassed patients with malignancy; patients with chronic diseases such as cardiovascular disease, liver cirrhosis, chronic kidney failure, and hematological disorders; patients with a history of autoimmune or currently undergoing immunosuppressant therapy; and patients with herpes simplex virus (HSV) co-infection. The sample selection involved purposive random sampling, with a minimum requirement of 60 subjects. The variables investigated within this study consisted of NLR and PLR as independent variables and CD4 as the dependent variable.

Laboratory Testing

The NLR and PLR were derived from routine hematology examination results using a hematology analyzer based on flow cytometry. The calculation of the NLR

involved dividing the total of neutrophils by the total of lymphocytes, while the PLR was determined by dividing the total of platelet count by the total of lymphocytes.

CD4 counts were also obtained using the flow cytometry method. All data were sourced from medical records, selecting patients who underwent both routine hematology test and CD4 test within a close period. For this study, a total of 80 patients were included.

Statistical Analysis

Statistical analyzes were conducted on both the main variables and patients' characteristic variables. The characteristics of the research subjects encompassed demographic variables such as age and gender, along with clinical variables including opportunistic infections, duration on ARV treatment, body mass index, total lymphocyte count, and hemoglobin. A normality test was conducted on continuous variables. Those that followed a normal distribution were presented as mean ± SD, whereas variables with a non-normal distribution were reported as median (interquartile range). Statistical analysis of categorical variables was conducted using the Chi-Square test, while continuous variables were analyzed using the Pearson test. The Pearson test was also performed to asses the correlation between NLR and PLR with CD4 count, and linear regression analysis was employed to identify the impact of NLR and PLR on CD4 count. To be considered as a statistically significant finding, the p-value should be <0.05.

RESULTS AND DISCUSSION

HIV Patients Characteristics

Table 1 shows the demographic and clinical profiles of research subjects. A total of 80 HIV patients were included. The majority of patients were ≥35 years old (53.8%), male (66.3%), had a normal BMI (51.3%), and had opportunistic infection (75%). Fifty-five (68.8%) of the patients had duration of ARV treatment for ≤3 months and 48 (60%) used tenofovir-lamivudine-efavirenz (TDF + 3TC + EFV) ARV regimen. Most of patients had CD4 count <200 cells/mm^3 (61.3%), had normal AST (71.3%), and had normal ALT (90%). Also, 44 (55%) patients were anemic.

Table 2 shows the hematological characteristics of the research subjects including neutrophils, lymphocytes, platelets, leukocytes, NLR, PLR, CD4, TLC, hemoglobin, AST, and ALT. The median CD4 at presentation was 103 (2 – 927) cells/mm^3, with the median NLR and PLR were 3.06 (0.87 – 15.94) and 181.03 (69.17 – 741.26), respectively.

Table 3 shows that opportunistic infection, body mass index (BMI), total lymphocyte count (TLC), duration on ARV treatment, and hemoglobin (Hb) were significantly associated with CD4 count.

Table 1. Demographic and Clinical Profiles of HIV Patients

Variables	Frequency (n)	Percentage (%)
Age		
<35 years	37	46.3
≥35 years	43	53.8
Sex		
Male	53	66.3
Female	27	33.8
BMI		
Underweight (<18,5 kg/m^2)	25	31.3
Normal (18,5 – 24,9 kg/m^2)	41	51.3
Overweight (≥25 kg/m^2)	14	17.5

CD4 count		
<200 cells/mm^3	49	61.3
≥200 cells/mm^3	31	38.8
Opportunistic infection		
Without opportunistic infection	20	25
With opportunistic infection	60	75
Candidiasis	19	31.7
Wasting syndrome	19	31.7
Pulmonary tuberculosis	6	10
Pneumonia	4	6.7
Toxoplasmosis	8	13.3
Cytomegalovirus	2	3.3
Pruritic papular eruption	4	6.7
Duration of ARV treatment		
≤3 months	55	68.8
>3 months	25	31.3
ARV regimen		
TDF + 3TC + EFV	48	60
TDF + 3TC + DTG	20	25
AZT + 3TC + NVP	10	12.5
TDF + FTC + EFV	1	1.3
AZT + 3TC + EFV	1	1.3
Liver function test		
Normal AST (5 - 40 µ/l)	57	71.3
High AST (>40 µ/l)	23	28.8
Normal ALT (7 - 56 µ/l)	72	90
High ALT (>56 µ/l)	8	10
Hematological characteristics		
Anemia (<13 g/dl in men, <12 g/dl in women)	44	55
Thrombocytosis (>450 × 10^3/µl)	4	5
Thrombocytopenia (<150 × 10^3/µl)	5	6.3
Lymphopenia (<1000 cells/mm^3)	29	36.4

Table 2. Hematological Characteristics of HIV Patients.

Variables	**Mean ± SD or Median (Range)**
Neutrofil (%)	65.15 ± 12.16*
Limfosit (%)	22.62 ± 10.07*
Platelet (10^3/µl)	287.43 ± 97.01*
Leukosit (10^3/µl)	6.5 (2.4 – 16.3)#
NLR	3.06 (0.87 – 15.94)#
PLR	181.03 (69.17 – 741.26)#
CD4 (cells/mm^3)	103 (2 – 927)#
TLC	1475.6 (3672 – 4661.8)#
Hb (g/dl)	12.25 (8.4 – 16.9)#
ALT (µ/l)	24 (8 – 190)#
AST (µ/l)	29 (15 – 208)#

*) Data with normal distribution are presented in mean ± SD

#) Data with abnormal distribution are presented in median (interquartile range)

Table 3. HIV Patients Characteristics Based on CD4 Count

Variables	CD4 Count				p-value
	<200 cells/mm^3		≥200 cells/mm^3		
	n	%	n	%	
Age					
<35 years	23	46.9	14	45.2	0.877[a]
≥35 years	26	53.1	17	54.8	
Sex					
Male	33	67.3	20	64.5	0.794[a]
Female	16	32.7	11	35.5	
Opportunistic infection					
Without opportunistic infection	4	8.2	16	51.6	0.000[a]
With opportunistic infection	45	91.8	15	48.4	
Duration on ARV treatment					
≤3 months	45	91.8	10	32.3	0.000[a]
>3 months	4	8.2	21	67.7	
Total lymphocyte count					
Normal	20	40.8	31	0	0.000[b]
Lymphopenia	29	59.2	0	100	
Hemoglobin					
Normal	15	30.6	21	67.7	0.000[b]
Anemia	34	69.4	10	32.3	
Body Mass Index (kg/m^2)					
Underweight	20	40.8	5	16.1	0.002[b]
Normal	21	42.9	20	64.5	
Overweight	8	16.3	6	25.8	

Bivariate analysis: a. Chi-Square; b. Pearson Correlation

This study found that the majority of characteristic variables of the patients were associated with CD4 count, except age and gender. This finding is consistent with the cohort study conducted in Cipto Mangunkusumo Hospital, Indonesia, which showed that age and gender were not linked with the increase of CD4 count (p = 0.112; p = 0.554).[12] A cohort study in Africa also proved that age was not a factor that significantly influenced the recovery of CD4 count after undergoing ARV therapy.[13] However, these findings are in contrast to the results of a cohort study in Iran, which revealed that older age affected lower CD4 count.[14] The aging process is linked with the atrophy of the thymus and reduced production of T and B lymphocytes.[15,16] Therefore, older patients can face a higher risk of HIV complications than younger patients due to weakened immune system.[14]

In terms of gender, the majority of the study participants (66.3%) were male. This is consistent with a research by Sajadipour et al.[17] , which found that males had higher rates of HIV infection than females because they engaged in riskier sexual behavior.[17] According to the Chi-Square test analysis results, there was no significant association between gender and CD4 count (p = 0.794). Similarly, study by Yogani et al.[12] also stated that gender was not related to an increase in CD4 count (p = 0.544).[12] In addition, a cohort study in Tanzania has proven that men and women have similar immunological and clinical conditions after one year of ARV treatment.[18]

This study displayed that most patients who have CD4 count lower than 200 cells/mm^3 had opportunistic infections. The bivariate analysis results using the Chi-Square test indicated a significant association

between opportunistic infections and CD4 count (p = 0.000). This result aligns with previous studies, that low CD4 levels were predictor of opportunistic infections.[19,20,21] Patients with CD4 <200 cells/mm^3 were shown to be 4.9 times higher of getting opportunistic infections than patients with CD4 >350 cells/mm^3.[19] The reduction in CD4 T lymphocytes can lead to impaired humoral and cellular immune responses, putting patients with low CD4 levels at risk of being more susceptible to various pathogenic infections.[22]

According to the duration of ARV treatment, it was found that the majority of patients with CD4 count <200 cells/mm^3 were patients undergoing therapy for ≤3 months, while those with CD4 count >200 cells/mm^3 were dominated by patients undergoing therapy for >3 months. The bivariate analysis using the Chi-Square test indicated a significant relationship between the duration of ARV treatment and CD4 count (p = 0.000). CD4 count can increase especially in the first 3 months after ARV initiation and continue to increase for up to 10 years of therapy.[23] This finding is also supported by study by Hidayat et al.[24] which found that ARV therapy for 6, 12, and 24 months had a significant effect on increasing CD4 levels in HIV patients.[24]

A cross-sectional study by Kwantwi et al.[14] in Ghana, West Africa revealed that total lymphocyte count (TLC), hemoglobin (Hb), and body mass index (BMI) could provide prognostic information about CD4 count in HIV patients.[25] Therefore, this study analyzed the relationship between TLC, Hb, and BMI with CD4 count. The analysis using the Pearson test showed that TLC, Hb, and BMI had a significant positive correlation with CD4 count. TLC has a strong positive correlation with CD4 count with a correlation coefficient of r = 0.767. Similar results were found in the study by Ola Wuan et al.[26] involving 121 HIV patients in Kupang, a strong positive correlation was found between TLC and CD4 count (r = 0.799).[26] This finding is also supported by several studies showing that TLC can be a predictor of CD4 levels.[27,28] The study conducted by Chen et al.[26] showed that TLC <1570 cells/mm^3 could be a predictor of CD4 levels <350 cells/mm^3 with a sensitivity of 65% and a specificity of 80%.[27]

This study found a moderate positive correlation between BMI and CD4 count (r = 0.422). Correspondingly, study conducted by Dwiadnyana et al.[29] showed a strong positive correlation between Hb and CD4 count (r = 0.698).[29] The occurrence of cytokine dysregulation, especially the increase in TNF, IL-6, and IFN-γ in HIV infection inhibits the process of erythropoiesis so that Hb levels can decrease along with decreased CD4 levels during the course of infection.[30] As a result, anemia is one of the hematological symptoms that occurs most frequently HIV infection.[31]

This study also found a weak positive correlation between BMI and CD4 count (r = 0.342). Matching results were found in the study of Kwantwi et al.[25], which stated a positive correlation between BMI and CD4 (r = 0.301).[25] Another research by Widiyanti et al.[32] also stated that the BMI value significantly affected increasing CD4 count.[32] BMI is an indicator for assessing nutritional status, which can be a predictor of immune status in HIV patients.[33] A study in China proved that HIV patients with higher baseline BMI had a better immune recovery process.[34]

Correlation between NLR and PLR with CD4 Count

As displayed in Table 4 and Figure 1, this study indicated a strong negative correlation between NLR to the CD4 count (r = -0.648; p = 0.000) and between PLR to the CD4 count (r = -0.668; p = 0.000).

Table 4. Correlation between NLR and PLR with CD4 Count.

Variables	Coefficient Correlation (r)	p-value
NLR	-0.648	0.000
PLR	-0.668	0.000

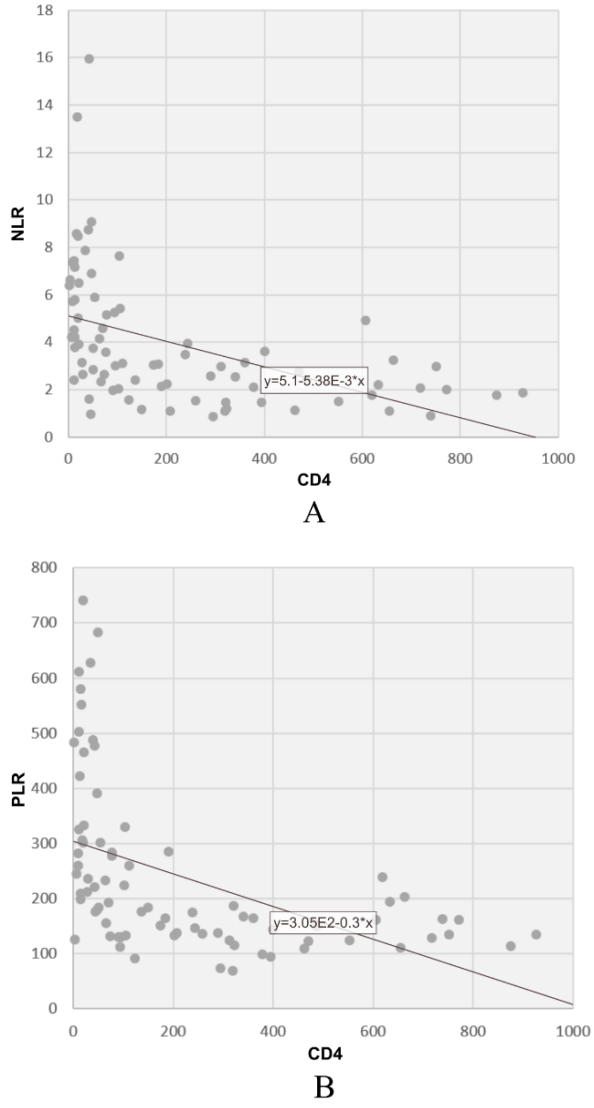

Figure 1. Scatter plot of the correlation between NLR and CD4 (A), PLR and CD4 (B)

Correlation between NLR and CD4 Count

Neutrophil-to-lymphocyte Ratio (NLR) refers to the ratio between neutrophils and lymphocytes which is used as a marker of the progression of various diseases including HIV. NLR combines both types of the body's immune response, lymphocytes as the adaptive immune response and neutrophils as the innate immune response. The Spearman correlation analysis between NLR and CD4 count yielded a correlation value of r = -0.648 which means that the higher the NLR value, the lower the CD4 count. This finding is in accordance with the research carried out by Handayani et al.[35], which found a negative correlation between NLR and CD4 count (r = -0.321; p <0.001).[35]

The discovery of a negative correlation between NLR values and CD4 count is backed by Nugraha dan Suryana's (2021) study on the association between NLR and CD4 with opportunistic infections.[36] The study proved that NLR has a positive correlation with opportunistic infections (r = 0.47; p <0.001), while CD4 has a negative correlation with opportunistic infections (r = -0.69; p <0.001).[36] Opportunistic infections are more common at higher clinical stages and lower CD4 levels.[19] Meanwhile, HIV patients with bacterial infection demonstrated a higher NLR compared to HIV patients who are not infected with bacteria due to an increase in neutrophils, especially during the beginning phases of the inflammatory response.[37] This shows that the increase in NLR occurs linearly with the decrease in CD4.

The opposite result was found in the study of Wande et al.[38] which showed that NLR had a weak positive correlation with CD4 (r = 0.375).[38] NLR values can be influenced by various factors including age, BMI, side effects of treatment for chronic infections such as hepatitis C and hepatitis B, and various chronic conditions, including cancer, diabetes, stroke, malnutrition, and coronary heart disease.[39,40] In addition, neutrophil levels can also decrease in advanced-stage HIV patients due to the cytotoxic effects of the virus and damage to hematopoietic stem cells which causes pancytopenia.[41] This decrease in neutrophil levels can cause a decrease in NLR values along with the decrease in CD4 count as HIV infection develops. Therefore, the use of NLR as a biomarker should consider the factors that can influence it, including medication usage and other medical conditions that affect hematology.

The standard NLR value typically falls within the range of 1 to 2.[40] Contrarily, an NLR value greater than three can indicate pathological conditions like cancer, inflammation, and infection.[40] Our study showed that the median of NLR was 3.06 (0.87 – 15.94) cells/mm^3, which means it has increased from the normal value. Correspondingly, research conducted by Emokpae et al.[42] also found higher NLR values in subjects with HIV compared to the control group.[42]

Increased NLR in HIV infection can occur from increased neutrophil or reduced lymphocyte counts. The increase in neutrophils happens due to basal hyperactivation of polymorphonuclear cells, driven by a greater release of proinflammatory cytokines, including IL-18, IL-22, TGF-B, and IL-8 during chronic inflammation.[43] On the other hand, the decrease in lymphocytes can occur from the decrease in CD4 T lymphocytes as a cytopathic effect of the virus through several mechanisms such as apoptosis, pyroptosis, or direct destruction by viruses.[5] Therefore, the increase in NLR in HIV infection occurs linearly with the decrease in CD4 count.

Correlation between PLR and CD4 Count

Platelet-to-Lymphocyte Ratio (PLR) is the ratio between platelets and lymphocytes which is used as an indicator to assess the progression of various diseases including HIV. A high PLR value can reflect the level of systemic inflammation and infection.[44] The Spearman test finding established a strong negative correlation between PLR and CD4 ($r = -0.668$; $p = 0.000$). This means that the higher the PLR value, the lower the CD4 level.

Although no studies have directly examined the relationship between PLR and CD4, previous study has discussed the prognostic role of PLR in HIV. The cohort study conducted by Raffetti et al.[9] demonstrated that an increasing PLR was linked with the mortality risk among HIV patients.[9] Using the Cox proportional hazard model, it was found that PLR <100 and PLR >200 compared to PLR 100-200 are linked with a higher risk of death.[9]

In HIV infection, increased PLR can occur due to increased platelets and decreased lymphocytes. Increased platelets occur due to dysregulation of various cytokines and coagulation biomarkers such as fibrinogen, fibrin, thrombin, D-dimer, and VWF.[45] Platelet activation levels can also increase due to the presence of viral antigen-antibody complexes and anti-platelet antibodies produced by B lymphocyte cells in response to viruses.[46] This was demonstrated in research conducted by Nkambule et al.[47] which showed higher platelet activation in HIV patients compared to the participants of the control group.[47] Meanwhile, advanced HIV infection can also have a cytopathic effect and induce increased apoptosis of CD4 T lymphocyte cells, causing lymphopenia.[48] Therefore, the increase in PLR in HIV infection occurs linearly with the decrease in CD4 count.

The Roles of NLR and PLR as Predictor of CD4 Count

The linear regression analyses concluded that NLR and PLR significantly influenced the CD4 count ($p = 0.020$; $p = 0.016$) as shown in Table 5. *Nagelkerke R Square* was 0.282 which means that the tested variable had a 28.2% influence on the dependent variable (CD4 count), while other variables outside this research analysis influenced the additional 71.8%.

Table 5. Linear Regression Test.

Variables	B	p-value	*Nagelkerke R Square*
NLR	-25.549	0.020	
PLR	-0.486	0.016	0.282
Constant	436.740	0.000	

The regression model in this multivariate analysis is as follows:

$$Y = 436.740 – 25.549\ X_1 – 0.486\ X_2$$

Y = CD4 count
X_1 = NLR
X_2 = PLR

Our finding in multivariate analysis showed that NLR and PLR had a prognostic role in determining the CD4 count. This result is strengthened by previous research which reported that NLR and PLR could be markers of the progressivity of HIV infection.[9,49] The cohort study conducted by Raffetti et al.[9] regarding the relationship between NLR and PLR with the mortality risk of 8230 HIV patients showed that patients with NLR 2-4 and >4 had a higher mortality risk compared to patients with NLR <2.[9] Also, patients with PLR <100 and PLR >200 had a higher mortality risk than those who had PLR 100-200.[9] Another cohort study also found that HIV patients with Non-AIDS-Defining Cancers (NADCs) who had increased NLR and PLR had a higher risk of death.[50]

A research conducted by Merriman et al.[51] involving 259 HIV patients, proved that increased NLR and PLR could arise among patients who were receiving ARV treatment or patients who were newly diagnosed with uncontrolled infections.[51] This study revealed that increased NLR was associated with patient mortality ($p = 0.0405$).[51] This corresponds with the study by Hanberg et al.[49] which demonstrated a strong relationship between NLR and PLR with patient mortality ($p < 0.0001$).[49]

An increase in NLR and PLR indicates systemic inflammation, which has been shown to increase the risk of mortality in various diseases.[9] Meanwhile, in HIV infection, CD4 count reflects the patient's immunological status and decreased levels of this marker is a factor that can increase the risk of mortality and morbidity.[52] This study identified a significantly strong negative correlation between NLR and PLR on CD4. This is corroborated by the result of the linear regression test, revealing that NLR and PLR contributed to a 28.2% influence on CD4 count. Thus, this study could validate that high NLR and PLR reflect the immunological status and the progressivity of HIV infection as indicated by low CD4 count.

STRENGTH AND LIMITATION

Our study highlights the impact of NLR and PLR in CD4 count and expands previous results about the prognostic role of these two markers. As inflammatory markers, they are cheap and widely available from routine hematology test in limited healthcare settings. This can provide a simple and easy way to determine the immune status of HIV patients as reflected by the CD4 count. There are several limitations such as we have not carried out serial monitoring of the hematological variables throughout the disease course and the data we collected consisted of patients who had varying durations of ARV treatment. Thus, we suggest a further study to investigate the predictive role of NLR and PLR in each disease stage with the larger sample size of HIV patients, which may provide more specific results.

CONCLUSIONS

This study found a significantly strong negative correlation between NLR and PLR to CD4 count in HIV patients, showing that high NLR and PLR could predict decreased immunity in HIV patients as indicated by low CD4 count. In health facilities where access to CD4 count testing is limited, NLR and PLR could serve as alternative prognostic parameters for monitoring treatment outcomes in PLHIV. This could possibly assist clinicians in monitoring HIV patients' immune status, thus contributing to a reduction in HIV-related morbidity and mortality.

ETHICAL CLEARANCE

This research was approved by the Health Research Ethics Committee of Dr. Moewardi Hospital with number 102/II/HREC/2023.

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CONFLICT OF INTEREST

We affirm that there are no conflicts of interest.

AUTHOR CONTRIBUTION

We affirm that all authors have contributed to this work. Each author has been involved in drafting and critically revising the content. All authors have given approval and agree to be accountable for this work.

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Original Article

Occupational and Return-To-Work Characteristics of Covid-19 Patients After Treated in Udayana University Hospital

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ABSTRACT

Corona virus disease 2019 (COVID-19) is a new disease caused by severe acute respiratory syndrome corona virus 2 (SARS-COV-2). The COVID-19's symptoms are fatigue, muscle pain, and psychological disorders. The purpose of this study was to describe the occupational characteristics and health conditions of COVID-19 patients who had recovered after being treated at Udayana University Hospital. This study is a descriptive study with a quantitative method and cross-sectional design. The research samples were 110 COVID-19 patients treated at Udayana University Hospital from June to August 2020 and taken using random sampling. The results showed that the highest proportion of respondents were aged between 24-44 years (44.5%), with almost equal proportions of women (50.1%) and men (49.09%). Most of them lived in Denpasar (46.36%). Most respondents work as private sector employees (24.55%), and 70% of them were using personal protective equipment (PPE) while working. Most respondents needed less than seven days to return to work after being declared "in recovery state" (60%), with the remaining 55.5% having a decreased work duration to be less than 8 hours per day. The proportion of respondents with comorbidities was 30.91%. As many as 27.27% were experiencing previously similar symptoms (fever, fatigue, cough) 4 to 5 months after being declared "cured." COVID-19 patients who have recovered should be monitored for a longer period of time to evaluate the symptom reoccurrence and its impact on their occupational and health conditions.

Keywords: Occupations, Back To Work, Comorbidity, COVID-19, and Quality of life.

Highlights: This study provides an overview of the characteristics of COVID-19 patients who have recovered in terms of work and health at the Udayana University Hospital.

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INTRODUCTION

Since December 2019, the world has been startled by the Chinese government's report on finding unusual pneumonia cases. In 2020, China confirmed the finding as COVID-19. COVID-19 is caused by Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2). This disease can cause pneumonia and severe respiratory distress like MERS and SARS. On March 11th, 2020, the WHO declared COVID-19 a pandemic after infecting 123 countries in Europe, Asia, America, and Africa. According to data from COVID-19 Task Force, up to March 20th, 2021, the number of confirmed positive COVID-19 cases has reached 124 million people with 223 countries infected, including Indonesia, since being found in Wuhan in December 2019.

Confirmed positive COVID-19 cases in Indonesia keep increasing, reaching 1.47 million people by January 23rd, 2021. From those numbers, 1.3 million people recovered, and 39,865 people died. Based on a study at several hospitals in Wuhan, of COVID-19 patients that had been declared recovered, 1038 out of 1655 respondents experienced fatigue and muscle pain, while 437 respondents experienced sleep disturbance. Meanwhile, 367 out of 1617 respondents had anxiety and depression. Patients with severe disease tend to experience lung diffusion and chest disturbance. These findings showed that patients that recovered from COVID-19 still might experience some physical or psychological symptoms.[1]

Based on those findings, we are interested in conducting this study to determine the occupational and return-to-work characteristics of patients treated at Udayana University Hospital in April-August 2020 after being declared recovered from COVID-19 for at least six months. Six months were taken as a cutoff for the respondents after declared recovered to minimize work-related activity affected by acute or long post-COVID phenomenon. Deep knowledge about these severe cases is expected to help clinicians in day-to-day practice in anticipating the worst possible outcomes during treatment.

MATERIALS AND METHODS

Methods

This study is a descriptive study with a quantitative method and cross-sectional design. The target population in this study is COVID-19 patients that recovered after being treated at Udayana University Hospital from June to August 2020. The sample size was determined using a sample size application by the WHO, resulting in 110 people.

Materials

Samples were taken using random sampling by accessing patients' medical records to determine which patients met the study criteria.[2] The inclusion criteria in this study were complete medical record data and the patient had completed education on how to fill the questionnaire. The exclusion criteria in this study were that the patient did not have device to support filling the google form, or illiterate patient. Data were collected using an online Google Form questionnaire sent to respondents by WhatsApp application. The collected data were presented as univariable to describe each variable's frequency distribution.

RESULTS AND DISCUSSION

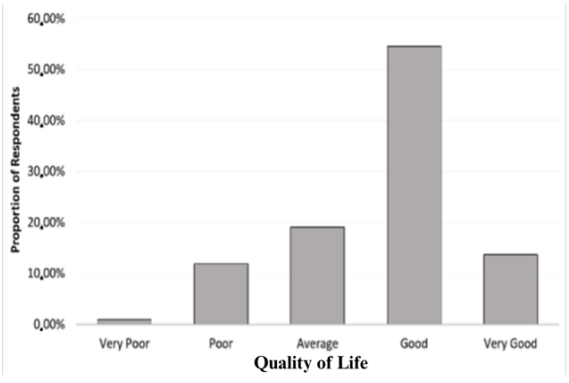

Figure 1. Respondents' Quality of Life.

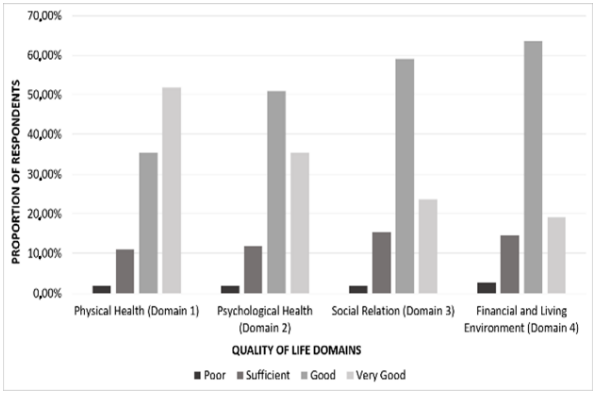

Figure 2. Respondents' Quality of Life Domain Distribution.

Respondents' Characteristics

A total of 110 respondents were included in this study. Respondents' characteristics, occupational characteristics, health conditions, and risky behaviors are shown in Table 1, Table 2, Table 3, and Table 4, respectively. Respondents' quality of life and quality of life domains are visualized in Figure 1 and Figure 2. Most (57,27%) were interviewed ten months after being declared recovered from COVID-19 (recovered in July).

Physical health domain was categorized as "poor" if the respondent experienced limitation in mild activities such as bending, kneeling, stooping, housework (e.g. carrying groceries, mopping the floor), or needed to routinely take medications for symptom relief, "sufficient" if the respondent experienced limitation in moderate activities such as moving table, climbing one flight of stairs, walking more than a kilometer, or occasionally needed to take medications for symptom relief, "good" if the respondent experienced limitation in vigorous activities, such as running, lifting heavy objects, or participating in strenuous sports, and "very good" if no significant limitation was experienced in doing physical activity, compared with the activity they used to do before diagnosed with COVID-19.

Psychological domain was assessed using several parameters, namely tiredness, feeling energized, peacefulness, nervousness, feeling worn out, and feeling downhearted/blue. Respondents were categorized as "poor" if the psychological parameters were causing them to take days off from their work, "sufficient" if respondents needed more time to finish their work compared than before diagnosed with COVID-19, "good" if they felt worse psychologically than before their COVID-19 diagnosis, but no significant effect on their work, and classified as "very good" if they never or only occasionally felt decrease in their psychological status after declared recovered from COVID-19.

In the social relation domain, respondents were assessed whether their physical and/or psychological problems interfered with their social activities such as visiting friends, relatives, attending social gatherings, and other social activities they normally used to do. They were categorized as "low" if the social problems were experienced most of the time, "sufficient" if the symptoms affected social life some of the time, "good" if the symptoms occasionally caused social issues, and "very good" if no social problems were experienced compared to before COVID-19 diagnosis.

For the financial and living environment domain, respondents were classified as "low" if they experienced significant decrease in their financial and/or living environment status, "sufficient" if they experienced moderate decrease, "good" if they experienced mild decrease, and "very good" if they experienced minimal or no issues in this domain.

Table 1. Respondents' Characteristics.

Characteristics	N	%
Age (mean ± SD)	(38.40±13.41)	
18-24 years	22	20.00
25-44 years	49	44.55
45-59 years	32	29.09
>60 years	7	6.36

Gender		
Male	54	49.09
Female	56	50.91
Location at the time of COVID-19 diagnosis		
Bangli	4	3.64
Badung	19	17.27
Buleleng	9	8.18
Denpasar	51	46.36
Gianyar	15	13.64
Jembrana	1	0.91
Karangasem	7	6.36
Klungkung	1	0.91
Tabanan	2	1.82
Outside Bali	1	0.91
Respondents' location after declared recovered from COVID-19		
Bangli	4	3.64
Badung	17	15.45
Buleleng	9	8.18
Denpasar	41	37.27
Gianyar	16	14.55
Jembrana	1	0.91
Karangasem	10	9.09
Tabanan	4	3.64
Outside Bali	8	7.27
Time gap between declared recovered until interview conducted		
9 months (recovered on August)	30	27.27
10 months (recovered on July)	63	57.27
11 months (recovered on June)	17	15.45

Table 2. Respondents' Occupational Characteristics.

Occupational Characteristics	N	%
Respondents' job before confirmed with COVID-19		
Not working	15	13.64
Governmental employee	19	17.27
Private sector employee	27	24.55
Hotel/Restaurant/ Commercial worker	1	0.91
Healthcare worker	6	5.45
Health professional	2	1.82
Farmer/fisherman/ market trader	5	4.55
Labor/daily worker	5	4.55
Self-employed/ Entrepreneur	14	12.73
Student/college student	11	10.00
Others	5	4.55
Duration of work per day before confirmed with COVID-19 (mean ± SD)	**(6.38 ± 3.15)**	
<8 hours	54	49.09
8 hours	46	41.82
>8 hours	10	9.09
Number of working days per week before confirmed with COVID-19 (mean ± SD)	**(4.88 ± 2.24)**	
0	16	14.55
1	1	0.91
2	2	1.82
3	3	2.73
4	25	22.73
5	46	41.82
6	17	15.45
7		
Respondents' job after recovering from COVID-19		
Not working	14	12.73
Governmental employee	18	16.36
Private sector employee	28	25.45
Hotel/Restaurant/ Commercial worker	1	0.91
Healthcare worker	6	5.45
Health professional	2	1.82
Farmer/fisherman/ market trader	5	4.55
Labor/daily worker	6	5.45
Self-employed/ Entrepreneur	13	11.82
Student/college student	11	10.00
Others	6	5.45
Duration of work per day after recovered from COVID-19 (mean ± SD)	**(6.10 ± 3.14)**	

<8 hours	61	55.45
8 hours	41	37.27
>8 hours	8	7.27
Number of working days per week after recovered from COVID-19 (mean ± SD)	**(4.71 ± 2.22)**	
0	17	15.45
2	1	0.91
3	2	1.82
4	8	7.27
5	27	24.55
6	42	38.18
7	13	11.82
Number of resting days before going back to work after declared recovered (mean±SD)	**(8.62± 8.87)**	
<7 days	66	60.00
8-14 days	23	20.91
15-21 days	13	11.82
22-28 days	4	3.64
>29 days	4	3.64
Gender characteristics of respondents who returned to work <7 days		
Male	41	62.12
Female	25	37.87
Age characteristics of respondents who returned to work <7 days		
18-24 years	12	18.18%
25-44 years	34	51.51%
45-59 years	18	27.27%
>60 years	2	3.03%
Gender characteristics of respondents who returned to work >29 days		
Male	3	75
Female	1	25
Age characteristics of respondents who returned to work >29 days		
18-24 years	0	0
25-44 years	0	0
45-59 years	2	50
>60 years	2	50
Usage of PPE while working		
Wearing PPE	77	70.00
Not wearing PPE	33	30.00
Type of PPE used while working (n=77)		
Face mask	76	98.70
Face shield	24	31.17
Medical latex gloves	22	28.57
Hair cap	14	18.42
Gown/special clothing	11	14.29
Protective boots/shoes	7	9.09

Table 3. Respondents' Health Conditions.

Health Characteristics	N	%
History of Comorbidity	34	30.91
Present	76	69.09
Absent		
Types of Comorbidity History (n=34)		
Pregnancy	2	5.88
Diabetes	8	23.53
Asthma	6	17.65
Cardiovascular	3	8.82
Renal failure	3	8.82
Nervous system disturbance	2	5.88
Cancer	1	2.94
Others (hypertension)	12	35.29
Re-experiencing symptoms after recovered		
Yes	30	27.27
No	80	72.73
Types of symptoms experienced after recovered (n=30)		
Fever	12	40.00
Sore throat	1	3.33
Cough	7	23.33
Flu	4	13.33
Shortness of breath	2	6.67
Nausea/vomiting	4	13.33
Diarrhea	1	3.33
Weakness	8	26.67
Headache	4	13.33
Loss of appetite	1	3.33
Neurological symptoms	1	3.33

Timing of symptoms Re-experienced after recovered (n=30)		
1 month	4	13.33
2 months	2	6.67
3 months	3	10.00
4 months	4	13.33
5 months	4	13.33
6 months	5	16.67
7 months	5	16.67
8 months	2	6.67
9 months	1	3.33

Table 4. Respondents' Risky Behaviors.

Risky Behavior Characteristics	N	%
History of Smoking		
Smoking	35	31.82
Not smoking	75	68.18
History of Alcohol Consumption		
Consuming alcohol	28	25.45
Not consuming alcohol	82	74.55
Travelling History		
Travelling	18	16.36
Not travelling	92	83.64
Travelling Destination (n=18)		
Buleleng	1	5.56
Denpasar	3	16.67
Jembrana	1	5.56
Karangasem	2	11.11
Klungkung	1	5.56
Outside Bali	10	55.56
Timing of travelling after recovered (n=18)		
2 months	2	11.11
3 months	1	5.56
4 months	1	5.56
5 months	2	11.11
6 months	2	11.11
7 months	5	27.78
8 months	2	11.11
9 months	3	16.67

This study found an almost equal proportion of male and female respondents. However, this result differs from other studies, where female patients were more common.[3,4] The majority of respondents were aged 25-44 years, considered a productive age group with a higher chance of being infected with COVID-19 due to their high mobility and frequent interactions. Almost all respondents lived in Bali, with the highest proportion living in Denpasar, with the highest number of COVID-19 cases in Bali. This finding is consistent with another study, where areas with higher population density and activity have higher COVID-19 cases.[3]

Occupational Characteristics

Most respondents worked as private sector employees before and after recovering from COVID-19. This finding was similar to another study, where the majority (30,67%) of respondents also worked as private sector employees.[5] As many as 70% of respondents were using PPE while doing activity in the workplace, with face masks as the most common PPE used (98,70%). Strict regulation in Bali might be the cause of this finding.[6]

Return-To-Work Profiles

Most respondents (60%) required less than seven days of rest before returning to work. Those whose aged 25 to 44 years dominated this group (51%). A higher proportion of males (62%) was found within this group. Whereas, the respondents who needed >28 days before returning to work were all aged 45 years or older, and most of them were men (75%).This finding is in line with a study conducted by Jacobsen et al.[8] that found women and older males had prolonged return to work. This might be related with other literature that stated males have more severe disease manifestations of COVID-19.[7,8]

Another study found that some patients experienced symptoms for over 28 days, even after being declared recovered.[8] In this study, almost half of the respondents worked less than eight hours per day (49.09%) before being diagnosed with COVID-19, which increased to 55.55% after recovery. In addition, there was a decrease in

the proportion of respondents working six days per week, from 41.82% before being confirmed positive to 38.18% after recovery.

The reduction in working hours and days may be caused by decreased health quality or regulations from their companies. However, it is also possible that factors such as employment status and government regulations may have affected the number of working days.

Health Characteristics

Several studies were conducted to investigate the impact of COVID-19 on the health and quality of life of patients who recovered from the disease. Results of these studies showed that hypertension was the most common comorbidity found in recovered patients[8–10], and individuals needed to control their blood pressure and pay attention to their lifestyle to prevent this condition.[10]

Additionally, many patients in this study experienced recurring symptoms after recovery, including fever, weakness, fatigue, and respiratory issues. This phenomenon, known as Chronic Post COVID-19, is common and emphasizes the need to practice health protocols to prevent re-infection.[1,11,12] Family and friend support is vital in boosting the patient's confidence and quality of life.[13-19]

Risky Behaviors

The study found that a significant percentage of COVID-19 patients had engaged in risky behaviors such as smoking (31.82%) and alcohol consumption (25.45%), which could increase their risk for severe disease.[15-17] The study also highlighted the importance of limiting travel to prevent the transmission of the virus, as almost all of the respondents did not travel to other regions (83.64%). [15,20]

The findings were consistent with previous studies, which showed that smoking[15,16,21] and alcohol consumption[17,22] could increase the risk of severe COVID-19 disease and that limiting travel is an essential preventive measure during the pandemic.[3,23-26] The study's results suggest that promoting healthy behaviors and limiting unnecessary travel could help prevent the spread of COVID-19 and maintain overall health.

STRENGTH AND LIMITATION

The study was conducted online using Google Forms, which might have limited the participation of those who do not have access to digital devices. Respondents may have had different interpretations of the questions, which could lead to bias in the study's results. Additionally, the study was conducted around six months after the patients recovered, which may have affected their recall of events and experiences, leading to recall bias. Finally, the questionnaire may also have had words or questions difficult for some respondents to understand, which could have caused further bias.

CONCLUSIONS

This study had almost equal proportions of male and female respondents, with an average age of 38 years. Many respondents were of adult age, lived in Denpasar, and worked as private sector employees. Most used PPE and needed less than a week to return to work after recovery. The highest comorbidity found was hypertension. Reoccurrence of symptoms was experienced by some respondents, with fever, weakness/fatigue, and respiratory problems being the most common symptoms. Most respondents did not smoke, did not consume alcohol, and did not have a traveling history. It is recommended that COVID-19 patients who have recovered should be monitored for a longer period of time to evaluate the possibility of symptom reoccurrence and its impact on their occupational and health conditions.

ETHICAL CLEARANCE

The research protocol was approved by Chairperson of the Research Ethics Commission, Faculty of Medicine, Udayana University with protocol number 2021.01.1.0612.

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CONFLICT OF INTEREST

The authors declared that no conflict of interest might bias or fabricate the information and work stated within the paper.

AUTHOR CONTRIBUTION

IMAW, MF and CAWP contributed to the proofreading and critically revised the article. IKJDK were responsible for data collection, analysis and interpretation of the data. IKJDK also wrote the article, and all authors, including IKJDK, IMAW, CAWP, MF, and HA gave final approval of the article.

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Original Article

In Silico Analysis of Inhibitor Potential of Punicalagin Compound in Pomegranate (*Punica granatum*) Against NS5 DENV-3 Protein

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ABSTRACT

Indonesia is one of the Dengue Virus (DENV) endemic areas which are dominated by DENV-2 and DENV-3. Until now, no specific drug therapy has been found to cure Dengue Virus Infection (DVI). Punicalagin is one of the active compounds that have the potential to be used as an antiviral. Unfortunately, not many studies have used punicalagin as a DENV antivirus. This study aims to determine the inhibitory potential of punicalagin compounds against NS5 DENV-3 protein through molecular docking. Molecular docking was performed using AutoDock Tools, ChemDraw, and Discovery Studio Visualizer. The target protein used is NS5 DENV-3 protein with PDB ID code: 4V0Q. The ribavirin compound was used as a positive control. The results obtained show that the punicalagin compound has the ability to attach to target receptors in the C-Terminal domain complex. This docking produces a bond free energy (ΔG) of -6.39 kcal/mol. This result is better than the ΔG of the control compound. Punicalagin's Inhibition Constant (Ki) value also showed better results than ribavirin. So it can be seen that the compound punicalagin effectively inhibits DENV replication and has the potential as a DENV drug candidate.

Keywords: Antiviral, DENV-3, *In Silico*, NS5 Protein, and Punicalagin.

Highlights: Add one short sentence of research's novelty and one short sentence of research's benefit.

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INTRODUCTION

Virus infection can occur to anyone and at any time, as it is still a global health problem.[1] One of the emerging virus is dengue virus (DENV) which can cause Dengue Virus Infection (DVI).[2] Dengue Virus (DENV) is a type of RNA virus that is transmitted through the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. This virus has four types of serotypes with a rapid spread throughout the world in recent years.[3]

WHO states that DVI has increased cases by 30 times worldwide in the last five decades. The distribution of DENV is faster in areas with tropical and subtropical climates.[4] Indonesia is a tropical country which has a high humidity level so that mosquitoes can survive in almost all parts of Indonesia.[5] The majority of areas in Indonesia are DENV endemic areas with DVI cases which tend to increase every year. This has resulted a health problem in Indonesia that is challenging to resolve.[6]

The data from the Ministry of Health of the Republic of Indonesia[7] noted that there were 108,303 DVI cases in 2020 with the four DENV serotypes circulating throughout Indonesia.[8] The results of a serological survey on the distribution of DENV serotypes in Indonesia stated that DVI cases in Indonesia were alternately dominated by DENV-2 and DENV-3. This change in dominance of the two serotypes is thought to have occurred due to the persistence or inheritance ability of the DENV serotypes in the main vector before being transmitted to humans.[9]

Currently, the handling of DVI cases focused on the development of antiviral drugs. This is an urgent need considering there is no specific drug therapy that is effective in inhibiting the growth of DENV. The development of a drug requires several stages of testing which takes a long time.[10] One of the early stages of drug development is the *in silico* testing through molecular docking. This test was carried out to determine the interactions that occur between the test compounds and the target receptors.[11]

So far, there have been many *in silico* studies to determine the antiviral activity of a compound to inhibit DENV replication. Secondary metabolites commonly found in natural products, such as quercetin, catechins, mangiferin, and arthemicin have been shown to inhibit DENV protein replication. This inhibition is based on the value of the Gibbs free energy (ΔG) with the highest inhibition value occurring in the mangiferin compound. The ΔG value represents the strength of the ligand binding to the receptor. The lower the ΔG value, the stronger the bond between the ligand and the receptor.[12] Several Indonesian herbal plants have also been tested to determine their ability to anti-DENV activity. The results of an *in silico* study conducted by Rosmalena et al.[13] stated that the artesunic acid and homoegonol compounds found in *Myristica fatua* have the ability to bind to the NS5 DENV protein complex with bond energies of -7.2 kcal/mol and -7.1 kcal/mol.

NS5 is the largest and most conserved protein complex (with more than 70% sequence identity among the four serotypes). The NS5 protein complex consists of two domains, namely the methyltransferase (MTase) domain at the N-terminal end and RNA-dependent RNA polymerase (RdRp) at the C-terminus. The high level of conservation in the NS5 protein structure makes it often used as a target for designing drugs with broad activity against several flaviviruses. The MTase domain (residues 1-265) plays a role in limiting viral RNA as well as N7 and 2'O ribose methylation activity. The RdRp domain plays a role in viral RNA replication. These two domains are connected by 5-6 residues (residues 266-271). The lack of activity of RdRp in host cells makes the NS5 complex a promising antiviral target for designing specific inhibitors with low toxicity.[14]

Punicalagin is a polyphenolic compound that is commonly found in pomegranate peels.[15] Punicalagin has been shown to have antiviral activity against the HSV-2 and SARS-CoV-2 viruses in silico. Until now, there has been no research regarding the effectiveness of punicalagin as a DENV-3 antiviral. Therefore, this study was conducted with the aim of knowing the potency of punicalagin inhibition against NS5 DENV-3 protein.

MATERIALS AND METHODS

Materials

The tool used for this research is a laptop device. The materials used for this study were three-dimensional files of punicalagin compounds and ribavirin compounds which were used as test and target ligands. The target receptor used in this study was the NS5 DENV-3 protein (PDB ID: 4V0Q).

Methods

This research was a descriptive observational study which aims to determine the ability of the punicalagin compound in pomegranates (*Punica granatum*) to bind the NS5 DENV-3 Protein using a pre-experimental one shot study design *in silico*.

Test of Physicochemical Properties

The physicochemical properties test refers to Lipinski's Five Laws or the Rule of Five. This test was conducted on the SwissADME website (http://www.swissadme.ch/).

Ligand Preparation and Optimization

Preparation begins by downloading the ligand and receptor structure data. Ligand structure data (punicalagin compounds and ribavirin compounds) are downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) in *sdf format. The downloaded data is converted to *pdb format using the ChemDraw Ultra application. The ligand optimization process was carried out in several stages including energy minimization, addition of H atoms, and addition of charge on the ligand structure. Ligand optimization was carried out using the AutoDock Tools and Chem3D Pro.

Receptor Preparation and Optimization

Receptor structure data (NS5 DENV-3 protein) was obtained through the Protein Data Bank database page (https://www.rcsb.org/) with PDB ID code: 4V0Q. Receptor preparation includes separation of native ligands and proteins, as well as other unnecessary molecules using the Discovery Studio Visualizer application. The final split result data is stored in *pdb format. Receptor optimization was carried out by adding H atoms and charges to the receptor structure using the Autodock Tools application. Optimization result data is saved in *pdbqt format.

Method Validation

Method validation was carried out by attaching native ligands to the protein structure using the AutoDock Tools application. The position of the grid box is placed at the midpoint of the ligand (X: 26.935; Y: 150.36; Z: 31.432) with dimensions X: 40; Y: 40; Z: 40. The method is said to be valid if the RMSD (Root Mean Square Deviation) value is below 2 Å.

Molecular Docking

The ligand binding process on the target protein was carried out using the AutoDock Tools application with the position of the grid box adjusted during method validation with dimension modification (X:126; Y:126; Z:126). The ligand binding process on the target protein will produce data and information that includes the bond interaction pattern formed,

the inhibition constant (Ki), and the bond free energy.

Visualization

The docking results are visualized using the Discovery Studio Visualizer application. The results are presented in the form of interaction patterns formed, inhibition constants (Ki), and bond free energy values (ΔG).

RESULTS AND DISCUSSION

Test of Physicochemical Properties

This test is based on the Rule of Five or Lipinski's Law of Five with 4 test parameters including the log P value, molecular weight, number of donor H atoms, and number of acceptor H atoms.[16] The results of the physicochemical properties of the test and control ligands are presented in the following table:

Table 1. Results of Test of Physicochemical Properties.

Test Parameters	Compound	
	Punicalagin	Ribavirin
Log P Value	-3,29	-2,94
Molecular Weight (g/mol)	1084,72	244,2
Number of H-Bond Donors	17	7
Number of H-Bond Acceptors	30	4

The results of the physicochemical properties test showed in Table 1 explained the punicalagin compound did not meet the 3 test parameters based on Lipinski's Fifth Law, while the ribavirin compound used as a control ligand fulfilled all the parameters of Lipinski's Fifth Law. Lipinski's Law of Five has 4 test parameters including Log P value <5, molecular weight <500 g/mol, number of H donors <5, and number of H acceptors <10. According to Lipinski's Rule of Five, a compound that has the potential to be used as a drug must meet the requirements for all parameters that have been determined.[17] This aura represents the level of capability of a compound to cross cell membranes.[16]

The Log P value is a parameter that shows the level of solubility of a compound in water or fat.[18] Compounds with a high level of hydrophobicity also have a high level of toxicity due to the inability of these compounds to penetrate the lipid bilayer and will spread widely in the body which results in a reduced level of selectivity of compounds for target receptors.[19] Log P values can still be tolerated at a ratio of -0.4 to 5.[20]

The molecular weight of a compound affects the permeability of a compound in penetrating the cell membrane. A compound having a molecular weight > 500 g/mol is unable to diffuse across the cell membrane. The number of hydrogen bond donors and acceptors is a test parameter in Lipinski's Fifth Law which aims to determine the number of hydrogen bonds needed for a compound during the absorption process.[18] The number of hydrogen bonds in a compound will be directly proportional to the amount of energy required during the absorption process.[21]

The process of designing a drug must be carried out carefully in order to avoid toxic effects and to optimize the effectiveness of the drug so that it can interact properly in the body. The physicochemical property test aims to minimize the toxic effects that arise from a drug on the basis of Lipinski's Five Laws. In addition, this law can also be used in predicting whether a compound can be given orally or not. Based on the 4 test parameters, the punicalagin compound did not meet the parameters of Lipinski's Fifth Law, while the ribavirin compound fulfilled all of the parameters of Lipinski's Fifth Law. A compound that does not meet the test parameters of Lipinski's Rule of Five cannot be administered orally. However, these compounds can still be given by injection.[20]

Method Validation

The method validation process is carried out before starting the belay process using the test compound. The docking method is acceptable and is said to be valid if the RMSD value obtained is less than 2.00 Å from the result of native ligand binding with the receptor.[22] RMSD (Root Mean Square Deviation) is a value that represents the relative deviation level when a ligand is tethered to the active site of the receptor.[19] The results of the method validation show that the method used is valid with an RMSD value of 1,659 Å. RMSD value < 2 Å indicates a stable bond between the ligand and the receptor. The smaller the RMSD value indicates the position of the atomic bonds in the ligand the better and closer to the original conformation.[22]

Molecular Docking

Molecular docking was performed with the AutoDock Tools 1.5.6 application. The test ligands used were punicalagin compounds, and ribavirin compounds as control ligands and glycerol as native ligands were used for comparison. The docking process was carried out 10 times to obtain the best conformation from the interaction between the ligand and the receptor. The docking results of the three ligands show different positions of the ligands and bonds formed. The binding positions of the three ligands are presented in the following figure:

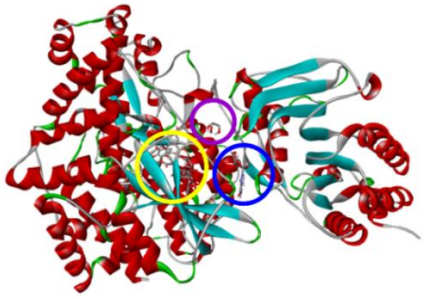

Figure 1. Binding Position of Test Ligand, Control Ligand, and Native Ligand on NS5 DENV-3 Protein.*

*Description: yellow: test ligand position, blue: control ligand position, purple: native ligand position

Figure 1 is the result of the binding of three ligands to the NS5 DENV-3 protein structure with different binding sites. The NS5 DENV-3 protein is a protein complex that plays a role in the DENV-3 replication process. This protein complex includes the largest protein complexes with the most durable protein components. The NS5 DENV-3 protein complex is composed of 900 amino acid residues which are divided into two active sites, namely the N-Terminal domain complex at residue range 1-262, and the C-Terminal domain complex at residue range 273-900.[23]

The NS5 DENV-3 protein complex is commonly known as a conserve protein. This is due to the important role of this protein complex in DENV-3 replication. For this reason, the NS5 protein complex is often used as a target receptor in *in silico* studies for the development of drug candidates.[24] Visualization of conserved proteins can be used to predict the bonds formed from the results of molecular docking (Figure 2).

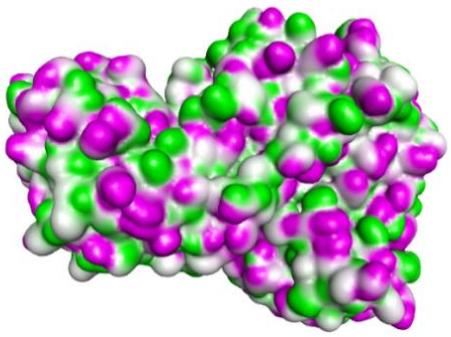

Figure 2. Representation of Conserved Protein on NS5 DENV-3 Protein.

Visualization of conserved protein NS5 DENV-3 shows the potential for 2 types of bonds. The green and purple colors in figure 3 represent the hydrogen bonds and hydrophobic bonds that can be formed in the NS5 DENV-3 protein complex. Hydrogen bonds and hydrophobic bonds are types of

bonds resulting from the interaction of the ligand with the receptor which play a role in maintaining the stability of the conformation of the ligand and receptor bonds.[25]

The interaction that occurs due to tethering of the test ligand on the target receptor produces hydrogen bonds and electrostatic bonds with amino acid residues in the range 340-737. This is different from the results of the binding of the control ligand which forms hydrogen bonds with amino acid residues in the range 67-582, and hydrogen bonds with amino acid residues in the range 300-355. The bond formed from the docking of the test and native ligands occurs in the C-Terminal domain complex of the NS5 DENV-3 protein, while the control ligand binds to amino acid residues in the N-Terminal and C-Terminal complexes.

The N-Terminal and C-Terminal complexes are protein complexes that play a role in the multiprotein replication process found in the NS5 DENV-3 protein. The N-Terminal complex has a methyltransferase enzyme that functions in the RNA translation phase into polyproteins in the host cell, while the C-Terminal complex contains an RNA polymerase enzyme that helps speed up the process of RNA replication.[23]

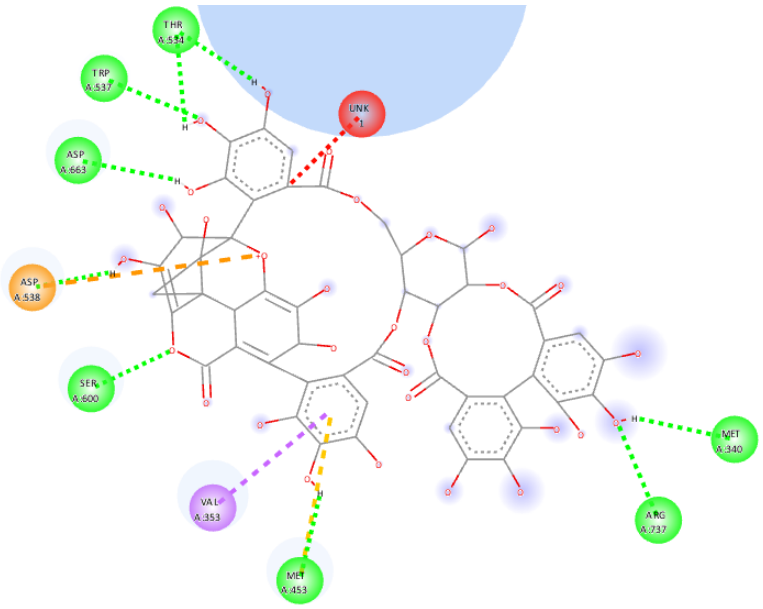

Figure 3. Bonds Formed by Molecular Docking of Test Ligand and Receptor.

Attachment of the test ligand to the target receptor results in three different types of bonds, namely electrostatic bonds (orange), hydrophobic bonds (purple), and hydrogen bonds (green) (Figure 3). These three bonds support the stable conformation of the ligand binding to the receptor. Electrostatic bonds are bonds that occur due to the distribution of electrons resulting in positive and negative charges on a molecule.[26] This type of bond helps to increase the conformational stability of the ligand bond with the receptor.[27] It also forms hydrogen bonds. Hydrogen bonds are said to be strong if they have a bond length above 1.85 Å.[28] This bond supports the stability of the protein structure.[29] Most of the hydrogen bonds formed from the interaction of the tested ligand and the receptor have a bond length of above 1.85 Å so that they have strong hydrogen bonds.

The hydrophobic bond formed from the interaction of the test ligand with the receptor also helps in reducing interactions with water molecules through alignment of the positions of non-polar compounds, thereby helping to maintain protein stability.[25] This interaction is formed with

the residue of the amino acid valine at point 353. Valine is a non-polar amino acid that is hydrophobic.[30] The binding position of the test ligand on the active site of the protein in the C-Terminal complex will interfere with the work of the RNA polymerase enzyme so that the process of viral replication cannot occur.

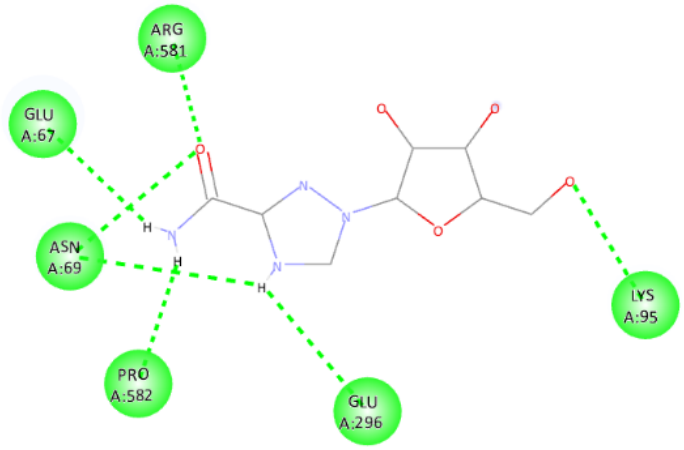

Figure 4. Bonds Formed by Molecular Docking of Control Ligand and Receptor.

Molecular docking of the control ligand with the target receptor produces only one type of bond, namely a hydrogen bond (Figure 4). The hydrogen bonds formed have a bond length above 1.85 Å. The strength of this bond contributes to maintaining the stability of the conformation of the ligand with the protein.[29]

The binding position of the control ligand in the C-Terminal and N-Terminal complex will interfere with the work of the RNA polymerase and methyltransferase enzymes so that the process of viral replication cannot occur.

Analysis of Molecular Docking Results

Molecular docking results were analyzed by comparing several data parameters, including inhibition constant (Ki), bond free energy (ΔG), and bonds formed from the docking process of test ligands, native ligands, and control ligands. This analysis was conducted to assess the level of effectiveness and potential of the tested ligands as drug candidates.

Table 2. Results of Molecular Docking.

Ligand	ΔG (kcal/mol)	Ki (μM)	Bond Type		
			Hydrogen	Hydrophobic	Electrostatic
Test	-6.39	20.67	MET340 MET453 THR534 TRP537 SER600 ASP538 ASP663 ARG737	VAL353	ASP538
Control	-6.09	34.52	GLU67 ASN69 LYS95	-	-

Native	-3.11	5.26	GLU296 ARG581 PRO582 LYS300 LYS355	-	-

The data presented in Table 2 is the best result from the 10x binding process of each ligand to the receptor. The inhibition constant (Ki) values of the three ligands have different values. The Ki value represents the level of strength of a compound in inhibiting the rate of action of the target receptor. The smaller the Ki value, the greater the inhibitory strength31. The test ligand Ki value was between the control and native ligand values. This shows that the inhibitory power of the tested ligands was lower when compared to the native ligands, but higher than the control ligands.

The bond free energy value (ΔG) is a value that indicates the degree of stability of the ligand conformation with the receptor. The ΔG value is inversely related to the level of affinity of the ligand for the receptor. The smaller the ΔG value, the greater the affinity of the ligand for the receptor.[22] Based on the results of the ΔG values of the three ligands, the ΔG value of the tested ligands was the best at -6.39 kcal/mol. This indicates that the bond of the test ligand with the target receptor is more stable than the control and native ligands. The binding free energy of the tested ligand is negative indicating that the tested ligand can interact with the receptor so that it can be used as a DENV-3 inhibitor. The magnitude of the ΔG value is influenced by several factors, such as differences in the number and types of bonds formed from the interaction of the ligand with the receptor[32], as well as the flexibility of the ligand structure during the binding process.[33]

STRENGTH AND LIMITATION

The strength of this research was that the punicalagin compound has a better binding energy than the control compound for the NS5 DENV-3 protein. This research was limited to computational tests only, so further *in vitro* tests are needed.

CONCLUSIONS

Based on the results obtained in this study, it can be concluded that the punicalagin compound is able to bind to the NS5 DENV-3 protein which is characterized by the presence of electrostatic bonds, hydrophobic bonds, and hydrogen bonds with amino acid residues in the C-Terminal domain complex which contains the RNA polymerase enzyme. The value of the inhibition constant of the punicalagin compound showed better affinity than the control compound, but lower than the ligand compound. Bond free energy (ΔG) values of punicalagin compounds showed the best results compared to native and control ligands. Therefore, the punicalagin compound is effective and has the potential to be used as a DENV-3 drug candidate.

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CONFLICT OF INTEREST

All authors in this research confirmed that there is no conflict of interest.

AUTHOR CONTRIBUTION

RK, SR, THS, MD, and AHR performed in charge of collecting data. RK writing article. YR is a principle investigator who provides study ideas and validates data.

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Original Article

The Effect of C-Reactive Protein Levels, Neutrophil, and Lymphocyte Count to Mortality of COVID-19 Patients with Sepsis in Referral Hospital

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by infection of Severe Acute Respiratory Distress Syndrom Coronavirus-2 (SARS CoV-2). COVID-19 patients may develop sepsis, the disregulation of the immune system that causes organ dysfunction and life-threatening situations. High mortality of COVID-19 and sepsis make it important to study. The purpose of this study is to analyze the effect of CRP levels, neutrophil, and lymphocyte count to mortality of COVID-19 patients with sepsis. This study is an analytic observational study with a cross-sectional approach. Samples were randomly retrieved of COVID-19 patients with sepsis admitted in referral hospital. Univariate, bivariate, and multivariate analysis used SPSS 26th version of Windows. The results of this study indicate a significant effect of CRP levels and neutrophil count on mortality of COVID-19 patients with sepsis. Meanwhile, lymphocyte count had no significant effects. The multivariate analysis showed its significance value. Partially, the effect of neutrophils on the patient's mortality has a significant value. The conclusion of this study is CRP levels and neutrophil count simultaneously have an effect on higher mortality of COVID-19 patients with sepsis.

Keywords: COVID-19, Sepsis, CRP, Neutrophils, and Lymphocytes.

Highlights: This study examined the relationship of the C-Reactive Protein (CRP) levels, neutrophil, and lymphocyte count to COVID-19 with sepsis cases multivariately.

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INTRODUCTION

Coronavirus disease 2019 or more commonly called COVID-19 is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) and it could be spread.[1,2] On March 11, 2020, COVID-19 was declared as a global pandemic.[3] Sepsis is the biggest problem that causes the mortality of COVID-19 patients. Abumayyaleh et al.[4] show that patients suffering from sepsis in COVID-19 had higher rates of comorbidity; 11% of COVID-19 patients fall into sepsis conditions based on the definition of Sepsis-3 International Consensus.[4] The sepsis itself is defined as the state of organ dysfunction caused by immune dysregulation to an infection. Infected pathogens can be bacteria, fungi, and viruses. This can lead to both danger and tissue damage. The high prevalence of sepsis, which is about 31.5 million sepsis patients worldwide makes it increasingly important to study. The extent of the organ dysfunction that is usually measured in terms of Sequential Organ Failure Assessment (SOFA) score causes a high mortality, 5.3 million people each year.[5,6]

C-reactive protein (CRP) is an acute phase protein released in response to infection. Increased release of IL-6 is also capable of stimulating the CRP secretion primarily produced by hepar cells.[7,8] Neutrophils constitute polymorphonuclear leukocytes that occupy the largest proportion of white blood cells in the body. Neutrophils become the first line of body defense against substance invasion.[9] Its activity is stimulated by increased proinflammatory cytokines. Lymphocytes are white blood cells responsible for controlling the adaptive immune system. If the number of T-lymphocyte cells is reduced, then there can be hyperinflammatory until death.

There have been studies that have studied the relationship between CRP levels and leukocytes of both mortalities and clinical severities of COVID-19 patients. Additionally, some studies also link it with sepsis patients' mortality. Based on research conducted by Seung Mok Ryoo et al[13], a significant correlation was found between the increase of CRP and the mortality of sepsis with a value of $p = 0.003$. Meanwhile, a study proved that CRP levels were higher in severe COVID-19 patients.[10] Leucocyte count has also been studied on deceased COVID-19 patients. Leucocytosis, neutrophilia, and lymphocytopenia are among the results. Of the deceased patients, most of them were COVID-19 in a severe stage.[11]

The COVID-19 prevalence is high and continues to have profound effects on life in this part of the world. Severe and critical patients of COVID-19 with sepsis have also contributed to a high mortality rate. According to previous studies, CRP levels affect clinical disseminations of both COVID-19 and sepsis patients. Moreover, the number of neutrophils and lymphocytes also affects the mortality of the COVID-19 patient. The previous studies examined such independent variables univariately either to COVID-19 patients or sepsis patients only so researchers are interested to study the effect of C-reactive protein levels, neutrophil, and lymphocyte count to mortality of COVID-19 patients - with depsis.

MATERIALS AND METHODS

The study is an observational analytic study with a cross-sectional approach. Research location is the Isolation Ward Dr. Moewardi Hospital, Surakarta, Indonesia. The actual population is COVID-19 patients with sepsis admitted to Dr. Moewardi's hospital in January until December 2021. The criteria for inclusion is a COVID-19 patient who was hospitalized at Dr. Moewardi surakarta in January - December 2021 and are over 18 years old, while the exclusion criteria are patients with immunodeficiency,

paraneoplastic syndrome, and patients taking immunosuppressant or steroid drugs. Samples were taken using the simple random sampling technique until it obtained 88 samples as a minimal number of samples and then an additional 10% so that 97 samples were used in the study.

CRP levels, neutrophil count, and lymphocyte count are the independent variables while the mortality is the dependent variable. Data analysis used the Statistical Program for the Social Sciences (SPSS) 26th version for Windows. The analysis used is univariate, bivariate, and multivariate. Bivariate used Spearman correlations test and multivariate used binary regression logistic test. The study has been approved by Dr. Moewardi's health research ethics commission and has obtained an ethical clearance number 819/VI/HREC/2022.

RESULTS AND DISCUSSION

Results

This study is an observational analytic study with cross-sectional approach. Data were collected from COVID-19 patients with sepsis. Table 1 below shows the characteristic of samples with distribution based on age and gender.

Table 1. Sample Characteristic.

Distribution	Total (n=97)	Outcome		Neutrophils count, mean ± SD	Lymphocytes count, mean ± SD	CRP levels, mean ± SD
		Survivor (%)	Non Survivor (%)			
Age (years)						
0-18	0 (0%)	0 (0%)	0 (0%)			
19-39	15 (15.46%)	3 (20%)	12 (80%)	10.34 ± 8.58	1.24 ± 0.77	12.35 ± 7.50
40-59	42 (43.30%)	19 (45.24%)	23 (54.76%)	8.64 ± 5.09	1.14 ± 0.44	12.68 ± 9.16
≥60	40 (41.24%)	12 (30%)	28 (70%)	9.38 ± 5.60	0.92 ± 0.40	12.56 ± 10.78
Gender						
Male	60 (61.86%)	24 (40%)	36 (60%)	8.80 ± 5.45	0.99 ± 0.48	13.78 ± 9.83
Female	37 (38.14%)	10 (27.03%)	27 (72.97%)	9.86 ± 6.60	1.18 ± 0.52	10.62 ± 8.87

Table 2. Outcome of COVID-19 Patients with Sepsis.

Sample		Frequency (%)	Neutrophil count, average ± SD	Lymphocyte count, average ± SD	CRP, average ± SD
Outcome	Survivor	34 (35.05%)	6.78 ± 4.01	1.15 ± 0.49	9.08 ± 7.84
	Non Survivor	63 (64.95%)	10.52 ± 6.36	1.02 ± 0.51	14.47 ± 9.91

Table 2. shows that the mortality of COVID-19 patient with sepsis was 63 patients from 97 samples. The table also mentions the average of each independent variable in both categories, survivor and non-survivor. Independent variables have been analyzed bivariously to know the correlation of each independent variable to the dependent variable. The significance of CRP levels is 0.005 and the significance of the neutrophil count is 0.001. At the same time, the significance of the number of lymphocytes is 0.151 (> 0.05). From the multivariate analysis, the determinant coefficient of the logistic regression is 0.193. From binary regression logistic test, the

significance value is 0.001 (<0.05) and thus it can be concluded that CRP levels and the number of neutrophils simultaneously affected the mortality of COVID-19 patients with sepsis.

Based on the binary regression logistic test, the significance of CRP levels is 0.098 (> 0.05) and the neutrophil count is 0.019 (< 0.05). From exp(b) it may be known as to the ratio of each independent variable. The exp(b) CRP levels are 1,047 and the variable neutrophil count is 1.150.

Discussion

CRP levels and mortality

The study found significant correlation (p= 0.005) between CRP levels and mortality of COVID-19 patients with sepsis. This coefficient correlation is positive, which means that the higher the CRP level, the higher the mortality rate of COVID-19 patients with sepsis. CRP levels have a 0.284 coefficient value of relations belonging to groups with weak correlation power. A study conducted by Wardika Sikesa [12] supports the results of the study. There is a significant CRP difference between a COVID-19 patient with moderate and severe symptoms. Thus, CRP levels affected the severity of COVID-19. In the same study, it has been suggested that there is a significant correlation between raising CRP levels to raising mortality in COVID-19 patients.[12] Seung Mok Ryoo et al.[13] conducted a sepsis related study and found a significant correlation between CRP increase and mortality of sepsis patients (p = 0.003).[13]

C-reactive proteins are acute phase proteins that are secreted more when inflammation occurs and reach peak values in 48 hours. Therefore, CRP can be used as an inflammation biomarker.[12–14] The course of COVID-19 disease with sepsis involves an inflammatory response. The rapidly increasing CRP is part of the first line defense of the body as an innate immune system enabled in order to fight off viral infections. At severe COVID-19, proinflammatory cytokines are oversecreted so as to affect CRP levels in the body. This extreme response can be harmful to the body because it leads to advanced organ dysfunction in a COVID-19 patient. The more severe the COVID-19 disease in patients, the higher the CRP rate.[12,15] Wardika and Sikesa [12] indicate that the highest CRP rate is owned by severe and critical COVID-19 patients[12] who fit the diagnostic criteria of sepsis.[16]

Neutrophil Count and Mortality

The study found a significant correlation (p= 0.001) between the number of neutrophils and the mortality of COVID-19 patients with sepsis. This relationship is positive, which means that the higher the number of neutrophils, the higher mortality rate of COVID-19 patients with sepsis. The coefficient value of the neutrophils count is 0.348, which means having moderate correlation power. Patients with COVID-19 with severe disease had significantly higher absolute neutrophil counts.[17] Sinurat et al.[18] mention the distinct number of meaningful neutrophils in COVID-19 degrees, mild, moderate, and severe. That is illustrated by the higher number of neutrophils in severe groups than those of mild degrees (4.3 vs 3.2 x 10^9/L).[18] Additionally, other studies cite the number of neutrophils measured within 24 hours after confirming diagnoses significantly correlated to COVID-19 mortality with a degree of significance of 0.002 (p< 0.05).[19]

The increasing number of neutrophils is due to some of the things described in the pathophysiology of COVID-19 that occur with sepsis. Once the SARS CoV-2 enters the body then infects the cell through its bound with the ACE-2 receptors, including the epithelial alveolar, it first activates the immune system. This is where the many neutrophils are activated as a body defense line against foreign invasion. Sepsis makes

the neutrophil's lifespan longer than normal conditions.[20] The rise in production of neutrophils is also set off by an increase in secretion mediator inflammation of viral infections, such as IL-6 and GCSF.[21] Neutrophil will make a neutrophil's extracellular traps (NETs) that acts to trap and kill the virus. Overdeveloped NETs, however, can harm the body by damaging lung tissue. In addition, neutrophils are also responsible for the formation of Reactive Oxygen Species (ROS) that can destroy the DNA of the cell and expel the virus from the cells.[18,22] Not only this, another mechanism for neutrophils is the direct destruction of the virus through Antibody Dependent Cell Cytotoxicity (ADCC).[18] The severe increase of neutrophils count can cause tissue damage and lead to poor outcomes.[23] At the state of sepsis, neutrophils can induce obstructive nasal paths that will cause mismatch and hypoxia conduction. Thus, neutrophils contributed to sepsis in ARDS.[24]

Lymphocyte count and mortality

The study results in that the number of lymphocytes and mortalities of COVID-19 patients with sepsis had no significant correlation (p= 0.151). As for the coefficient value of correlation, the lymphocytes count of 0.174 means having weak correlation power. Negative value of coefficient correlation means lower levels of lymphocytes do not significantly affect mortality levels. Some studies support these results. The study mentioned that there is no correlation between the number of lymphocytes and the mortality of sepsis patients (p= 0.465).[25] In addition, a diagnostic test of COVID-19 patients mentioned that lymphocytes had poorer diagnostic marks in COVID-19 patients than the number of neutrophils and NLR.[26]

Characteristics of the immune suppression on sepsis conditions is apoptosis of T-helper, cytotoxic lymphocytes, B lymphocytes, and dendritic cells.[27] Other reference mentioned that. in the case of sepsis. there will be an increase in the neutrophil count followed by an increase in the lymphocyte count. In turn, the number of lymphocytes may develop apoptosis if sepsis is not properly handled.[28] In a study from Martins et al. [29], lymphocytes count were significantllower in patients with sepsis than the control group y.[29] In COVID-19 patients with sepsis they can either increase or decrease the number of lymphocytes. Some samples in the study also showed high levels of lymphocytes in COVID-19 patients with sepsis who passed away in either 48 hours or more. Thus, according to analysis data of this study, the number of lymphocytes does not significantly affect the mortality of a COVID-19 patients with sepsis (p= 0.151).

However, there are other studies that contradict those results. Tarigan et al.[19] analyzed the correlation of lymphocyte count measured in the first 24 hours of COVID-19 patient mortality and had significant results (p= 0.002; P < 0.05).[19] Other research also revealed that sepsis patients who have persistent lymphopenia were at a risk of dying by 5.66 times greater than being non persistent lymphopenia.[30] The differences in previous studies with these may be due to some factors. One is the patient's comorbidity, which has not been analyzed in this research. Based on a statistical analysis, comorbidity contributes more to affecting mortality than the number of absolute lymphocytes.[21]

CRP levels, neutrophil count, lymphocyte count, and mortality

The contribution of independent variables (CRP levels, neutrophil count, and lymphocyte count) to the dependency variable is 19.3%. CRP levels in a partial way have no significant impact on mortality while the number of neutrophils has a significant partial influence on mortality. Based on exp(b) ratio, patients with increasing CRP rates have a mortality risk of 1,047 times greater than those with low CRP levels. Meanwhile, patients with an increasing

number of neutrophils will have a mortality risk of 1,150 times greater than those with a low number of neutrophils.

No previous study has analyzed multivariately the number of neutrophils, the number of lymphocytes, and the CRP concentrations together in COVID-19 patients with sepsis. Previous studies analyzed the independent variable as Neutrophil Lymphocytes Ratio (NLR). The study from Nurhayatun et al.[31] showed that NLR increase in COVID-19 patients increased the risk of death.[31] Both neutrophils and CRP levels play a role and are directly involved in the human body's defense systems when there is both infection and inflammation. Previous studies have proved that each of these variables has significant correlation to the severity of both COVID-19 and sepsis and affects its mortality. Hyper-activated neutrophils lead to the formation of overloaded NETs and ROS results a danger condition to the body.[18] In addition, neutrophils have a tendency to induce ARDS in sepsis patients.[24] CRP as a biomarker inflammation increases as the rate of inflammation increases. Increased CRP leads to increased risk of organ dysfunction and death in sepsis. The study proved that CRP levels and the neutrophil count simultaneously affected COVID-19 patients' mortality with sepsis.

STRENGTH AND LIMITATION

The strength of this study was the novelty of research about COVID-19 with sepsis and using a multivariate analysis. The limitation of this study was no analysis about patient comorbidities.

CONCLUSIONS

The increase in CRP levels and the neutrophil count simultaneously have an effect on higher mortality of COVID-19 patients with sepsis. The variable that has the most influence is neutrophils because in multivariate analysis neutrophils have significant partial influence value. Meanwhile the lymphocyte count had no significant correlation to mortality of COVID-19 patients with sepsis. For future research relevant to this study, comorbidities of the patients need to be analyzed beside the independent variables.

ETHICAL CLEARANCE

The research protocol was approved by Dr. Moewardi's health research ethics commission and has obtained an ethical clearance number 819/VI/HREC/2022.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

ANM collected and analyzed data, wrote the manuscript and generated the figure and tables. DRH proofread the manuscript and revised the manuscript. AS proofread the manuscript. EN designed this study.

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Case Report

Exploring the Therapeutic Potential of Glycyrrhizic Acid in Liver Implication in Dengue Infection: A Case Report

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ABSTRACT

Dengue is one of the most common infectious diseases affecting humans. The virus is transmitted between humans by the *Aedes* mosquito. It occurs hyperendemically in tropical and subtropical climates worldwide. Dengue infection can affect numerous organs, with the liver being the most frequently affected organ. The clinical spectrum of liver disorders ranges from mild elevation of transaminase enzymes to severe conditions such as acute liver failure. Several mechanisms have been proposed to describe hepatic dysfunction observed in dengue fever and dengue hemorrhagic fever, such as immunological injury, hypoxic injury, and direct viral damage due to reduced hepatic perfusion during shock. Glycyrrhizic acid, extracted in the form of glycyrrhizin from the root of the licorice plant *Glycyrrhiza glabra*, is referred to as Stronger Neo-Minophagen-C (SNMC®). It has shown effectiveness in reducing serum aminotransferase and bilirubin levels, attenuating hepatocyte apoptosis, and producing endogenous interferon. The following is a case report of a 23-year-old woman with dengue fever and elevated liver enzyme level. The patient's vital signs were stable. A physical examination revealed no abnormalities. A complete blood count test showed thrombocytopenia without an elevation of the hematocrit. AST level was 901 U/L after admission. Causes of other hepatitis infections, such as hepatitis A, B, and C, were excluded. The dengue IgM and IgG antibody levels were reactive. After several days of hospitalization, the patient experienced clinical improvement after supportive therapy and the administration of glicyrrhizic acid or SNMC®.

Keywords: : Dengue Infection, Elevated Liver Enzyme, Glycyrrhizic Acid, Hepatic Dysfunction, and SNMC®.

Highlights: This report highlights the use of glycyrrhizic acid in the prevention of acute liver failure in dengue infection with liver involvement.

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INTRODUCTION

Dengue fever, an acute infectious disease transmitted between humans by the *Aedes* mosquito, and is caused by dengue virus (DENV). This RNA virus belongs to the genus Flavivirus and family Flaviviridae, with four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Although these serotypes share some antigenic similarities, their significant differences lead to the provision of cross-protection for only a limited period following infection with any one among the four. Secondary infections with a distinct serotype or the occurrence of several infections involving other serotypes can initiate extreme forms of dengue.[1,2] Furthermore, dengue infection occurs hyperendemically in tropical and subtropical climates worldwide, particularly in urban and semi-urban regions.[3] The occurrence of dengue has shown a substantial surge, experiencing an eight-fold increase over the last two decades. The cases climbed in 2000 from 505,430 to exceeding 2.4 million in 2010 and further escalated to 4.2 million by 2019.[3,4]

Dengue can affect numerous organs, with the liver being the most frequently affected. The clinical spectrum of liver disorders ranges from mild elevation of transaminase enzymes to severe conditions such as acute liver failure.[5] Several mechanisms have been proposed to describe hepatic dysfunction observed in dengue fever and dengue hemorrhagic fever, such as immunological injury, hypoxic injury, and direct viral damage due to reduced hepatic perfusion during shock.[6–8]

Glycyrrhizic acid, extracted in the form of glycyrrhizin from the root of the licorice plant Glycyrrhiza glabra, is referred to as Stronger Neo-Minophagen-C (SNMC®) by Dexa Medica in Indonesia. It is a triterpene glycoside majorly comprising flavonoids, hydroxyl coumarins, and β-sitosterol, alongside glycyrrhetinic acid, which has various pharmacological and biological activities.[9] Additionally, it is effective against viral hepatitis, specifically chronic viral hepatitis, and is capable of stimulating endogenous interferon production.[10] Glycyrrhizic acid derivates were reported to have anti-dengue activities by conjugating with amino acids. The introduction of aromatic acyl hydrazide residues into the carbohydrate part also strongly influenced on the antiviral activity of glycyrrhizic acid against DENV2.[11] In vitro analyses by Crance et al.[12] using human hepatoma cells demonstrated that glycyrrhizin could inhibit hepatitis A virus penetration, probably by changing the fluidity of the cell membrane.[12] Moreover, glycyrrhizin can help reduce elevated liver enzyme levels by inhibiting phospholipase A2 activation and controlling changes in hepatocyte membrane[13] permeability, which represses the production of hepatitis B surface antigen (HBsAg).[14]

This report presents a case of dengue fever in a 23-year-old female with liver implications, without any evidence of viral hepatitis infection. The focus of this discussion centers on the diagnosis and management of the liver affected by dengue infection.

CASE DESCRIPTION

A female aged 23 years presented at the emergency department with a primary complaint of high-grade fever persisting for three days, accompanied by myalgia and retro-orbital pain. Nausea and vomiting occurred four times daily, while spontaneous bleeding, such as epistaxis and gingival bleeding, was not reported. There was no history of previous illnesses, including hepatitis, diabetes mellitus, or allergies. No family members exhibited similar symptoms, and the patient had not recently traveled to another city.

Upon admission, consciousness was observed, along with the following vital signs: blood pressure (BP) 100/60 mmHg,

pulse per minute at 100 beats, respiratory rate per minute at 18 breaths, oxygen saturation at 98% in room air, as well as 37.7°C body temperature. Physical examination revealed petechiae in the upper extremities and mild tenderness in the right upper quadrant and epigastric region. Laboratory tests indicated 14.0 g/L (11.7-15.5 g/L) hemoglobin (Hb), 39% hematocrit (Hct); white blood cell count (WBC), 5.1x10^3/µL, platelet count (PC) of 32x10^3/µL, alanine aminotransferase (ALT), 255 U/L (normal < 35); aspartate aminotransferase (AST), 901 U/L (normal < 35); and 123 mmol/L (135–147) serum sodium. Hepatitis markers were all negative, while clinical suspicion of dengue fever was verified by positive anti-dengue antibodies (IgM and IgG), as detailed in Table 1.

Table 1. Laboratory Test During Admission.

Examination	Result
Blood cell count	
Hb	14 g/dL
Hct	39%
WBC	5.1 × 10^3/µL
PC	32 × 10^3/µL
Blood chemistry	
AST	901 U/L
ALT	225 U/L
BUN	35 mg/dL
Creatinine	0.66 mg/dL
Glucose	85 mg/dL
Na	123 mmol/L
K	4.4 mmol/L
Cl	97 mmol/L
Immunoserology	
IgM anti-HAV	Negative
IgG anti-HAV	Negative
HBsAg	Negative
Anti – HCV	Negative
IgM anti-dengue	Positive
IgG anti-dengue	Positive

Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell; PC, platelet count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; IgM, immunoglobulin M; IgG, immunoglobulin G; HAV, hepatitis A virus; HCV, hepatitis C virus.

Chest radiological examination showed no abnormalities, while abdominal ultrasound indicated achalculous cholecystitis (blue arrow) are shown in Figure 1 and 2.

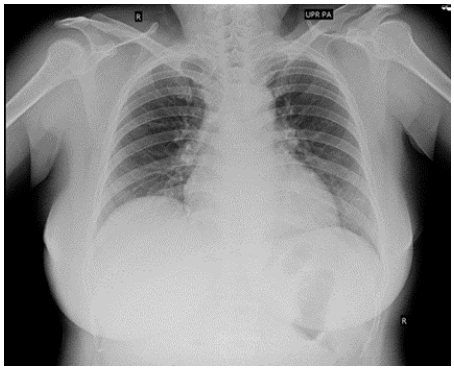

Figure 1. Chest X-Ray of The Patient.

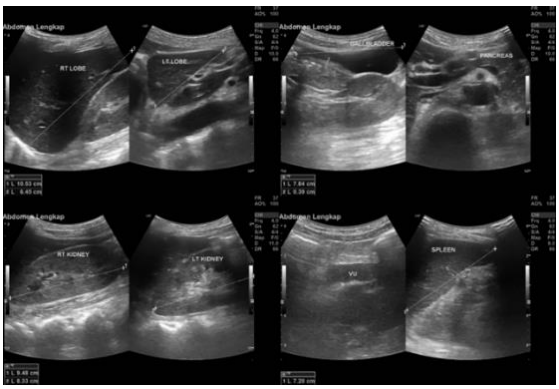

Figure 2. Abdominal ultrasound of the patient.

A diagnosis of dengue fever with liver involvement was established. The patient received appropriate fluid therapy and symptomatic medication, along with glycyrrhizic acid infusion of two ampules daily for five days to correct serum sodium levels. The complete blood count (CBC) was monitored every 24 h, and liver enzyme levels were evaluated after completion of the infusion as detailed in Table 2.

Table 1. Laboratory Test During Hospitalization.

	Day of hospitalization								
Markers	0	1	2	3	4	5	6	7	8
Hb	14	10.7	10.5	11.3	11.3	10.9	11.0	11.8	11.5
Hct	39	30	30	32	32	30	32	33	33
WBC	5.1	3.8	4.0	4.4	5.0	4.9	4.8	5.1	5.5

PC	32	26	34	27	36	42	65	89	120
AST	901				806				88
ALT	285				282				56
Na	123		134						
K	4.4		3.8						
Cl	97		108						

*) Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell; PC, platelet count; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Following five days of glycyrrhizic acid infusion, the liver enzyme levels showed slight improvement. The infusion was continued for an additional five days, with the addition of curcumin, containing curcumin and lysin, three times daily. After a total of 10 days, the liver enzyme levels exhibited significant improvement, and the patient was asymptomatic, leading to discharge.

DISCUSSION

The diagnosis of dengue fever in the patient was confirmed based on the criteria set by the WHO in 2011, including sudden onset of fever within 2-7 days, presence of petechiae, platelet count <150,000/mm^3, and absence of plasma leakage signs. Additionally, the observed malaise symptoms such as headache, body aches, and retro-orbital pain were consistent with the conventional manifestations of dengue infection.[2] The identification of acute primary infection through positive results for dengue IgM antibodies further confirmed the diagnosis of dengue.

Defective liver function in the patient was attributed to dengue fever infection, considering the negative consequences of hepatitis virus marker examinations. When a person has a dengue infection, their liver dysfunction can range from minor (as indicated by an increase in aminotransferases alone) to severe (as indicated by jaundice and even fulminant hepatic failure).[15]

This was in line with existing studies showing that the liver was repeatedly affected by dengue fever.[5] Hepatitis is discovered in 60-90% of dengue fever cases, characterized by mild to moderately elevated transaminase levels almost five times above the normal value. Meanwhile, severe hepatitis, characterized by transaminase levels surpassing 10 times the upper limit of normal, is only encountered in a mere 3-11% of cases.[16] The distinctive feature of liver cell damage is the elevation of ALT levels over AST levels, distinguishing it from liver damage caused by the hepatitis virus.[5,17]

The liver damage pathophysiology in dengue remains incompletely comprehended but is generally related to interactions between the host, viruses, as well as the time of disease. Hepatocytes and Kupffer cells are primary viral targets.[5] The virus attaches to the hepatocyte cell surface receptor, and protein E plays a crucial role in this process. Sulfate sulfur is also recognized to facilitate DEN virus entry into liver cells, referred to as HepG2. Liver cells in the G2 phase are sensitive to the disease, enabling viral multiplication. Subsequently, liver lesions, microvesicular steatosis, apoptosis, as well as the appearance of Councilman-Rocha Lima bodies, comparable to the conditions in yellow fever as well as different hemorrhagic viral infections are presented.[18,19] Liver damage may result from direct viral effects on hepatocytes, as previously described, or due to disruptions in the response of the host immune system toward the virus; additional factors contributing to liver injury include ischemia or hypoxia in hepatocytes initiated by circulatory disorders. Furthermore, drug administration, including paracetamol or acetaminophen, which are frequently used to manage fever or discomfort in dengue, tends to promote liver damage.[5,16]

No specific therapy is available for hepatitis in dengue cases; however, the primary treatment objectives focus on viral clearance, seroconversion, and reducing inflammation.[20] In contrast to viral hepatitis, achieving viral clearance and seroconversion in dengue hepatitis treatment is not feasible, making mitigation of inflammation the primary target. Glycyrrhizin is known to have anti-inflammatory, antioxidant, and hepatocyte membrane-stabilizing properties.[20] Additionally, it exhibits anti-hypertransaminase effects by disrupting the release of transaminase enzymes into the bloodstream of patients with liver parenchymal damage or inflammation, namely, hepatocyte necrosis.[21] SNMC® in Indonesia is available in the form of ampoules (20 ml) comprising 40 mg of glycyrrhizin, 400 mg of glycine, and 20 mg of L-cysteine (BPOM).

In case of dengue fever, SNMC® was administered at two ampoules (40 ml) for a total of eight consecutive days. This is in accordance with the recommended dosage displayed in the package insert, which suggests 40-60 ml but does not exceed 100 ml daily (BPOM). The observed advancement in liver function was similar to the significant enhancement in transaminase activity reported by Lin et al (2015) after administering 100 ml of SNMC® for five days in a case of acute exacerbation of hepatitis B.[22]

STRENGTH AND LIMITATION

The strength of this study is that it demonstrated the effectiveness of SNMC® as an anti-inflammatory agent in liver involvement in dengue infection. A limitation of this study is that future large-scale studies are required.

CONCLUSIONS

In conclusion, this case report shows the potential efficacy of glycyrrhizic acid treatment, known as SNMC®, in managing dengue hepatitis. However, there is a need to conduct further extensive and well-designed research focusing on the use of SNMC® in dengue hepatitis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Data curation, writing–review and editing, and validation: ISP. Data curation, supervision, and validation: PR.

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Original Article

Relationship between Knowledge and Stigma with Attitude Towards People with Leprosy in Professional Nursing Students

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ABSTRACT

The bacteria *Mycobacterium leprae* is the source of the chronic infectious illness leprosy (*M. leprae*). In society, leprosy still carries a shame. The erroneous impression of leprosy gives birth to stigma. Leprosy is thought to be brought on by curses, witchcraft, divine retribution, sin, or genetics. A person's perception of leprosy and lack of understanding about it might have an impact on how they feel about those who have it. Even among health students, information alone will not be sufficient to end the stigma against those who have leprosy; also, students need to learn how to develop greater empathy for those who have the disease. This study sought to ascertain the association between leprosy knowledge and stigma and attitudes among nursing students at the professional level. In this study, a cross-sectional methodology is used with a descriptive correlational design. A total 320 professional nursing students participated in the survey. Total sampling was used to select respondents based on inclusion and exclusion criteria. Utilizing the SPSS version 21, data were gathered by questionnaire and analyzed using the Spearman's rho test at a significance level of 0.05. The Spearman's rho test results revealed a positive link between attitude and knowledge (p=0.001), but a negative relationship between attitude and stigma (p=0.000). It was determined that attitudes toward people with leprosy were significantly influenced by information, stigma, and those attitudes. The better the mindset, the more one knows about leprosy. Leprosy patients are treated better when there is less stigma associated with their condition.

Keywords: Leprosy, Knowledge, Stigma, Attitude, and Nursing Student.

Highlights: As a development of the Precede Proceed behavioral theory from Laurence Green (1980), the results of this study are expected to have a positive impact on developing the concept of nursing science regarding nursing care management.

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INTRODUCTION

Leprosy is a systemic disease and has a pre-examination of skin and nerves.[1] Leprosy is a chronic infectious disease caused by the obligate intracellular *Mycobacterium leprae* (*M. leprae*) which attacks the peripheral nerves of the skin as the first affinity, then the skin and oral mucosa, respiratory tract and spreads from the upper part to other organs except the central nervous system.[2] Leprosy is also a disease that is still a stigma in society. This stigma arises because of wrong perceptions of leprosy. Many people think that leprosy is a disease caused by curses, witchcraft, divine punishment, sin, food, or heredity.[3] Even in the area of health students, education is not enough to suppress the stigma and negative attitudes toward lepers, so knowledge about how to increase empathy for lepers must be added[4]. According to research conducted by Raju and Kopparty in the National Leprosy Eradication Program (NLEP) in India, knowledge does not necessarily eliminate stigma and negative attitudes towards leper.[5] This illustrates the low awareness and negative attitude toward leprosy.[6] Indonesia is one of the countries with leprosy cases that are still stable due to the decrease in the number of cases. East Java is the largest contributor to leprosy cases in Indonesia. Also according to research conducted by Rufina[7], the relationship between the level of knowledge and the stigma of Hansen's disease in USU Medical Faculty students is very low. And according to a study conducted by Da Silva and Paz[4] entitled Nursing Care Experiences with Hansen's Disease Patients: Contributions from Hermeneutics, there is still stigma and discrimination against people with leprosy from health professionals.[4]

In 2017, the highest distribution of new cases of leprosy in Indonesia by province population of 100,000 occurred in East Java with 3.373 new cases.[8] Based on data from east Java Health Office, until January 21st 2020 there were 2,668 new lepers found. Meanwhile, 3.351 lepers were still in treatment; 255 of them infected children arrived at stage 2 or disabled and 194 children were at stage 1. A preliminary study conducted by the researcher on February 1st 2020, by interviewing nursing students at Airlangga University from 14 respondents selected randomly obtained data such as nursing professional students know what leprosy is and leprosy transmission. However, there is still a negative perception of lepers (stigma) and from the results of preliminary study it can be concluded that they will refuse to visit the house of lepers, besides refusing to buy food from a former leper and finally there is an opinion that they will stay away from the person affected by leprosy. From the statement, it has impact on lepers because nursing professional students as prospective health workers should be responsive and always care for patients as well as giving good attitudes toward the desires of patients who want to get treatment; however, it is feared that they will not provide optimal services.[9]

Knowledge is the result of human sensing or the result of knowing about an object through sensory organs such as eyes, nose, ears, etc.[10] Meanwhile, stigma is a negative name for a person or group so that it is changes their self-concept and social identity.[2] The impact of stigma on the lives of leprosy clients occurs in four domains: emotions, thoughts, behavior and relationships.[11] Knowledge and stigma in nursing professional students at the professional stage have a role in determining how is their attitudes toward lepers. Meanwhile, attitudes are a readiness or willingness to act, and not an implementation of a particular motive. Attitudes are not an action (open reaction) or activity, but a predisposition to behavior (actions) or closed reaction.[12] In a study conducted on medical students at Saint James School of Medicine, Bonaire, Dutch Caribbean in 2015, it was found that knowledge of Hansen's disease among first, second, and third semester

students was higher than that of fourth semester students [13]. This is thought to be due to the factors of forgetting what they have learned about the disease in the third semester.[12]

The researcher intends to do research on the professional stage of nursing students who are prospective health workers who will later face various patients including lepers and is also supported by the absence of research on professional students on the stigma and their attitudes toward leprosy sufferers.

In addition, it is a development of the theory of *Precede* Proceed by Laurence Green[14] which states that human behavior is influenced by behavioral factors and factors outside of behavior applied in nursing as a service to individuals, families, and communities.[14] The results of this study are expected to have a positive impact on developing the concept of nursing science about nursing care management. In the field of nursing itself, it is contained in the Indonesian Nursing Professional Standards, which is included in the area of caregiving and nursing care management in core competencies, as nurse graduates are able to compile nursing plans and take nursing actions according to the plan.[15]

From the introduction described above, researchers are interested in examining the relationship between knowledge and stigma with the attitudes of students at the nursing professional stage toward leprosy.

MATERIALS AND METHODS

Study Design

This research design used a correlational design with a cross-sectional approach. The cross-sectional approach is carried out by identifying and measuring only once at a time without any follow up.[16]

Population

The population of this study were nursing students who were in the professional stage at the national universities in East Java, such as Airlangga University, Brawijaya University, and Jember University. In this study, researchers used total sampling, a sampling technique where the number of samples is the same as the population.[17] The number of samples in this research is 320 people.

Variables

This research variable measures the level of knowledge about leprosy, the stigma of leprosy sufferers and attitudes toward lepers.

Instruments

The instrument in this research for knowledge variables used knowledge questionnaire from Asmaradianty's research modified by the researcher,[18] while stigma variables used the Explanatory Model Interview Catalogue (EMIC) stigma scale, and for attitude variables used the Social Distancing Scale (SDS).[19,20] In measuring attitudes, SDS was chosen because it measures the level of social distance; a high score indicates a person's high tendency to maintain social distance from sufferers.[21]

Statistical Analysis

This research used Spearman's rho test analysis with significance level $\alpha = 0,05$ in SPSS software version 21.

RESULTS AND DISCUSSION

Results

Table 1. Distribution of Demographic Characteristics of Respondents, The Relationship Between Knowledge and Stigma and Attitudes Toward Leprosy among Nursing Students at The Professional stage.

Respondent Demographic Characteristics	Category	Frequency	Percentage (%)
Sex	Male	53	16.6
	Female	267	83.4
	Total	**320**	**100**
Location	Airlangga	111	34.7
	Brawijaya	61	19
	Jember	148	46.3
	Total	**320**	**100**
Age	22 years	23	7.19
	23 years	236	73.75
	24 years	61	19.06
	Total	**320**	**100**

Table 1 is a distribution of demographic characteristics of respondents. It shows that from a total of 320 respondents spread from three national universities in East Java, almost all respondents have female gender (267 people or 83.4%) and almost half of the respondents came from the University of Jember (148 people or 46.3%). In addition, almost all respondents were 22 years old (236 people or 73.75%).

Table 2. The Relationship Between Stigma and Attitude Toward Lepers in Nursing Students at The Professional Stage.

Stigma	Attitude				Total		P-Value	*r*
	Negative		Positive					
	f	%	f	%	∑	%		
Low	6	4.9	117	95.1	123	100	0.000	-0.286
Medium	29	18.1	131	81.9	160	100		
High	15	40.5	22	59.5	37	100		

Table 2 shows the relationship between stigma and attitude toward lepers in nursing students at the professional stage. The results of the analysis of the relationship between stigma and attitude show that the stigma level of nursing students in the professional stage toward leprosy show that the majority of respondents (160) have a moderate stigma level and the attitude of nursing students in the professional stage toward leprosy show that most respondents (270) have a good attitude level. Almost half of the respondents (131, 81.9%) have a moderate stigma against lepers with a positive attitude. The results of further statistical tests obtained the value of p = 0.000 (p <0.05), it can be concluded that H1 is accepted, so that there is a relationship between stigma and attitudes toward lepers among nursing students at the professional stage.

Discussion

The results of statistical tests using Spearman's rho show that there is a significant relationship with the level of correlation being at a very weak level between knowledge and attitude. In addition, the correlation coefficient shows that the correlation coefficient is positive, which means that the higher the knowledge, the better the attitude shown by the professional stage nursing students toward lepers.

Knowledge is an important factor in determining attitudes toward lepers. The results of this study indicate that the majority of respondents (259 respondents) have high knowledge and the majority of respondents (270 respondents) have a good attitude, which can be seen from the aspect of trust toward lepers. This is the same as research conducted by Britton[22] which states that nurses are equipped with knowledge while still in education and believe that a person will not easily contract leprosy if they have treated lepers well.[22] It is also corresponds with the theory that someone's knowledge will adopt a new behavior.[10]

The results of the respondents' answers distribution analysis on to the knowledge variable showed that the mode of respondents with most number of correct answers was on the disability aspect of leprosy and the most number of answers was wrong on the aspect of leprosy transmission. This is slightly different from Sharma's[23] research in India to second, third, and fourth year medical students with the highest scores being those who answered correctly about the causes of leprosy, while the lowest scores were those who answered correctly about the pathology of leprosy.[23] The respondents answers distribution analysis to the attitude variable showed that the mode of the respondents was mostly willing to answer the emotional aspects of life, such as showing an attitude of not objecting to being neighbors with people with leprosy.

According to this result, it can be said that the high level of knowledge possessed can make someone behave well. Education is a process of changing a person's behavior and attitudes. Besides that, one of the factors that influence human behavior is the knowledge factor itself. It was found that the high knowledge possessed can encourage someone to have good behavior. The results of statistical tests using Spearman's rho show that there is a significant relationship with the level of correlation being at a weak level between stigma and attitude. In addition, the correlation coefficient shows that the correlation coefficient is negative or inverse, which means that the lower the level of stigma, the better the attitude shown by professional nursing students toward lepers. It is the same with the theory that stigma is formed from stereotypes or beliefs in something. Stereotypes are beliefs about certain groups. Stereotypes can be positive or negative and stereotypes in lepers are they are seen as disgusted by the clinical manifestations seen from the type of leprosy they have and the disability that has been experienced. Thus, the stigma about leprosy that is owned can affect the attitude toward lepers.

The results of this research indicate the majority of respondents (160 respondents) gave medium stigma. One of the causes of stigma is belief about the cause of stigma where trust itself is a component of attitude. A stigma example of leprosy patients is that they are seen as disgusted because of the clinical manifestations that appear from the type of leprosy suffered and the disability experienced. Stigma can encourage prejudice against a person or group of people.

It is slightly different from the research conducted by Singh[24] in the community in Nepal; according to the results of the study, the majority of the respondents (44%) have a high stigma about lepers, supported by myths and misconceptions that exist in the community about leprosy.[24] The results of this research show that the majority of respondents (270 respondents) have good attitude toward lepers. This contrasts with other case studies of chronic diseases such as HIV/AIDS; people with HIV/AIDS report receiving bad care by health workers. Patients with chronic diseases who are stigmatized as bad also report that health workers feel frustrated with them, complain, and treat them differently or unfairly.[25]

The results of the respondents' answers distribution analysis to the stigma variable

found that the most respondents' mode of answering was probably located in the prejudice aspect, while the mode that had the least answer was yes, which was located in the stereotype aspect. This is the same as research conducted by Kaehler[26] on people in Thailand which showed that 49.8% said they would not buy food from lepers because of the fear of contracting leprosy and also negative perceptions about leprosy. In addition, the difficulty of lepers in finding work is also supported by research conducted on leprosy sufferers in Nolombo where leprosy sufferers are required to leave work because of their illness.[26]

In addition, some of the factors that influence attitude of leprosy patients in terms of the quality of management leprosy are: the high social stigma of leprosy in the community and among health workers, which hinders case finding and management of leprosy; the community does not know the early symptoms of leprosy; most leprosy control program holders are not doctors; comprehensive management of leprosy (including prevention of disability) is not optimal; leprosy clinically resembles many other skin diseases, so supporting examinations are needed. While supporting examination facilities for diagnosis are not yet available in all healthcare facilities, the leprosy journey is very long so that reactions that arise after treatment are not monitored.[27]

It can be said that the level of owned stigma can make a person behave well. Owned stigma can change a person's behavior and attitudes. In addition, one of the causes of stigma is belief about the causes of stigma, where belief itself is a component of attitude. According to this statement, it was found that the level of stigma possessed could encourage a person's good behavior.

STRENGTH AND LIMITATION

The strength of this study was that it is known that the level of stigma possessed can make a person behave well. The stigma possessed can change a person's behavior and attitude. In addition, one of the causes of stigma is a belief about the cause of stigma, where the belief itself is a component of attitude. It has been found that the level of stigma possessed can encourage a person's good behavior. The limitation of this study was the Covid-19 virus pandemic which caused all research processes to be carried out online.

CONCLUSIONS

According to the results and discussions of this research, it can be concluded:

1. Knowledge of leprosy has a relationship with attitudes toward lepers. Knowledge of leprosy has a positive relationship with attitudes toward lepers and the two variables have a very weak relationship.
2. The stigma of leprosy has a significant relationship with attitudes toward lepers. The stigma about lepers has negative relationship with the attitudes toward lepers and the two variables have a weak relationship.
3. In this research, it was found that the high knowledge of leprosy which is obtained by nursing students at the professional stage during education can deal with the stigma and bad attitudes of nursing students at the professional stage of leprosy.

ETHICAL CLEARANCE

This research has passed the ethical approval of the Health Research Ethics Commission of the Faculty of Nursing, Airlangga University with ethics certificate number 1994-KEPK.

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CONFLICT OF INTEREST

There are no conflicts of interest between authors in this study.

AUTHOR CONTRIBUTION

Ishomatul Faizah: Study design, methodology, software, data collection, writing of the original manuscript. Laily Hidayati, Ika Nur Pratiwi: Study design, methodology. All authors read and approved the final manuscript.

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Review Article

Re-Emergence of Ampicillin Sensitive *Salmonella* Typhi and the Increase of Ciprofloxacin Resistance in Typhoid Fever Treatment in Asia: A Systematic Review

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ABSTRACT

Typhoid fever is a disease caused by *Salmonella* Typhi infection. In 2000, 2.16 million people were affected worldwide, with more than 90% morbidity and mortality in Asia. Ampicillin is the first-line antibiotic used for typhoid management. However, the rise in resistance to first-line antibiotics has shifted ciprofloxacin as an alternative. This study aimed to describe the trends in ciprofloxacin- and ampicillin-resistant *Salmonella* Typhi in Asia. This study was a systematic review that conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria. Search was indicated toward studies on *Salmonella* Typhi susceptibility toward ciprofloxacin and ampicillin were identified using PubMed, Cochrane Library, and ProQuest. Of the 1542 studies found, only 16 fulfilled the criteria. In 1996, *Salmonella* Typhi was not found to be resistant to ciprofloxacin, whereas 3.5% was resistant to ampicillin. In 2005, ciprofloxacin resistance increased to 19.3%, whereas ampicillin resistance decreased to 13.3%. In 2005-2014, a larger number of *Salmonella* Typhi isolates resistant to ciprofloxacin than to ampicillin. Furthermore, during 2016-2019, resistance to ciprofloxacin increased from 8.1% to 95%, while ampicillin resistance increased from 27.5% to 85.2%. This the high ampicillin resistance in South and East Asia. In Asia, there was an increase in ciprofloxacin-resistant *Salmonella* Typhi from 1996 to 2019, whereas ampicillin-resistant *Salmonella* Typhi decreased from 1996 to 2015. Between 2016 and 2019, contrasting evidence was found in East Asia and South Asia, where resistance toward ampicillin increased.

Keywords: *Salmonella* Typhi, Ciprofloxacin, Ampicillin, Susceptibility, and Resistance.

Highlights: In Asia, there was an increase in ciprofloxacin-resistant *Salmonella* Typhi, while a corresponding decrease was observed in ampicillin-resistant *Salmonella* Typhi.

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INTRODUCTION

Typhoid fever is a serious disease that can threaten life. It is caused by *Salmonella enterica* serovar Typhi (*Salmonella* Typhi).[1–3] There were 2.16 million typhoid cases in the world in 2000, where 90% of its morbidity and mortality occurred in Asia. According to the WHO, five countries in Asia, China, India, Indonesia, Pakistan, and Vietnam, are endemic countries for typhoid fever. However, South Asia incidence is significantly higher than Southeast and Northeast Asia.[4]

First-line antibiotics consist of ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (co-trimoxazole).[5,6] On the other hand, the inappropriate uses of antibiotics caused an increased number of multi drug resistant (MDR) and extensively drug resistant (XDR) *Salmonella* Typhi. MDR is defined as *S.* Typhi that is resistant to first-line antibiotics[7,8] while, XDR is defined as *Salmonella* Typhi that is resistant to fluoroquinolone, third-generation cephalosporin, in addition to first line antibiotics.[9]

Ampicillin is one of the first-line antibiotics against typhoid fever that is often used due to several factors, such as easy access, relatively cheap price, and fewer side effects.[10] Although chloramphenicol reduces fever more quickly, it has notable side effects such as suppression of bone marrow that can lead to bone marrow aplasia and death.[11,12] Therefore, ampicillin is preferred over chloramphenicol.[13]

Chloramphenicol was used as first-line antibiotic against typhoid since 1948; however, increasing resistance and side effects gave rise to increased ampicillin and co-trimoxazole use.[14] Improper use of first-line antibiotics led to the emergence of MDR in the 1960s.[9] Fluoroquinolone was then used as alternative due to the emergence of resistance toward first-line antibiotics.[15] The universal use of fluoroquinolone caused emergence of resistant strains.[16] A study conducted in Cambodia in 2008-2015 reported that, over time, there was a decrease of ciprofloxacin susceptibility from 100% to 93.1% and a decrease in the number of MDR from 62.9% to 17.2%.[17] Another study conducted in India, reported a decrease in ampicillin resistance from 53% to 23% in the course of time after the use of fluoroquinolone, cephalosporin, and azithromycin.[18] This fluctuating trend of *Salmonella* Typhi antibiotic resistance, in addition to the high morbidity and mortality of typhoid fever in Asia, encourages the need to evaluate further the overall trends of ciprofloxacin resistance and sensitivity to ampicillin in Asia. This study aimed to describe the ciprofloxacin and ampicillin resistant *Salmonella* Typhi in Asia.

METHODS

Eligibility Criteria

This study was a systematic review that was made based on PRISMA criteria. This review used data from studies that have been published from 1991-2021 regarding *Salmonella* Typhi susceptibility to ciprofloxacin and ampicillin in Asia. The exclusion criteria were studies designed as case report, review, or systematic review, study with only title or abstract as well as the following: used language other than Indonesian or English, could not be accessed, did not show data of interest, did not specify Salmonella as *Salmonella* Typhi, used methods other than disk diffusion for antibiotic susceptibility test, and did not identify year or location.

Literature Searching

Literature search was done in May 2022 on PubMed, Cochrane, and ProQuest with keywords: "*Salmonella* typhi OR *Salmonella* enterica serovar Typhi OR salmonella typhosa AND ciprofloxacin OR ciprofloxacin hydrochloride OR Ciprofloxacin Hydrochloride Anhydrous

AND ampicillin resistance OR Ampicillin Resistances OR Resistance, Ampicillin OR Resistances, Ampicillin". The search was set to obtain studies from 1991-2021.

Study Selection and Data Extraction

Search results from each database were collected with Zotero to remove duplicates. Next, title and abstract were screened based on inclusion and exclusion criteria defined. The data from studies included that were extracted are : first author, publication year, experiment year, study area, numbers of sample tested, specimen type, numbers of resistant isolates per year, antibacterial resistance testing method, and interpretation criteria.

The main outcomes in this review, which are the number of resistant isolates and total isolates per year tested with the disk diffusion method, were combined, and converted into percentages. These percentages were then made into bar charts grouped for each geographical region (Asia, South Asia, South-East Asia, and East Asia).

Risk of Bias Assessment

All selected studies were assessed for risk of bias using "Critical Appraisal Tools for Use in Joanna Briggs Institute Systematic Reviews for quasi-experimental studies." Study was included if they fulfilled more than 50% of the criteria.

RESULTS AND DISCUSSION

Search Results and Study Characteristics

Out of 1542 studies that were collected from the databases, 72 were excluded for duplicates. A total of 1470 studies were screened by reading the title and abstract. Further screening was carried out resulting in 208 full-text articles. As many as 78 studies could not be assessed, 73 studies did not show data of interest, 23 studies did not specify Salmonella as *Salmonella* Typhi, 12 used methods other than disk diffusion for antibiotic susceptibility test, and six studies which showed unidentified year or location were excluded (Figure 1). All these screenings and selections resulted in 16 studies included and assessed for their quality using JBI. JBI consisted of nine criteria, assessing if the study: 1) showed clearly the cause and effect from the study; 2) similar participants included in any comparisons; 3) participants included in any comparisons received similar treatment or care; 4) studies had a control group; 5) there were multiple measurements of the outcome both pre and post the exposure; 6) the follow up was complete; 7) outcomes of participants included in any comparisons were measured in the same way; 8) outcomes were measured in a reliable way; 9) and appropriate statistical analysis were used. These criteria were answered with yes, no, unclear, or not applicable (Table 1). More than 50% of all questions were answered with "yes" in all the studies, resulted in 16 studies for further analysis.

The studies included were published from 1998-2020 and covered several Asian countries, six from India[19–24], three from Nepal[25–27], two from Bangladesh[28,29] , two from Pakistan[30,31], two from Indonesia[32,33], and one from Iraq[34]. Specimens tested consisted of 7162 blood and 56 feces samples. Susceptibility testing methods used were Kirby Bauer or disk diffusion. All our studies used Clinical & Laboratory Standards Institute (CLSI) or National Committee for Clinical Laboratory Standards (NCCLS) as the interpretation criteria.

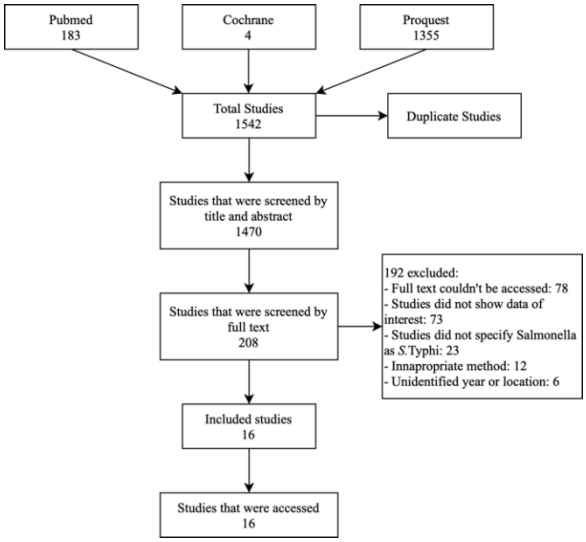

Figure 1. PRISMA Flowchart. PRISMA Diagram That Were Made During Article Searching From May 2022 until July 2022.

Table 1. Quality Assessment for Risk of Bias using JBI Criteria.

No	Study	1	2	3	4	5	6	7	8	9	Conclusion
1	Khadka et al.[25]	✓	✓	✓	✓	✓	✓	✓	✓	✓	Included
2	Ali et al.[28]	✓	✓	✓	✗	✓	✓	✓	✓	?	Included
3	Alam et al.[19]	✓	✓	✓	?	✓	✓	✓	✓	?	Included
4	Shah et al.[30]	✓	✓	✓	?	✓	✓	✓	✓	✓	Included
5	Ahmed et al.[29]	✓	✓	✓	✓	✓	✓	✓	✓	✓	Included
6	Lugito et al.[32]	✓	✓	✓	?	✓	✓	✓	✓	✓	Included
7	Khanal et al.[26]	✓	✓	✓	?	✓	✓	✓	✓	✓	Included
8	Mohanty et al.[20]	✓	✓	✓	✓	✓	✓	✓	✓	?	Included
9	Kumar et al.[21]	✓	✓	✓	✓	✓	✓	✓	✓	✓	Included
10	Saeed at al.[31]	✓	✓	✓	?	✓	✓	✓	✓	?	Included
11	Khanal et al.[27]	✓	✓	✗	?	✓	✓	✓	✓	?	Included
12	Sharvani et al.[22]	✓	✓	✓	✓	✓	✓	✓	✓	?	Included
13	Dutta et al.[23]	✓	✓	✓	✓	✓	✓	✓	✓	?	Included
14	Kumar et al.[24]	✓	✓	✓	?	✓	✓	✓	✓	✓	Included
15	Al-Mayahi et al.[34]	✓	✓	✓	✓	✓	✓	✓	✓	?	Included
16	Amdani et al.[33]	✓	✓	✓	?	✓	✓	✓	✓	?	Included

✓=question answered with "Yes", ✗= question answered with "No", ? = question answered with "Unclear"

Trend of *S.* Typhi Resistance to Ciprofloxacin and Ampicillin

There were 16 studies that reported *Salmonella* Typhi resistance to ciprofloxacin and 13 studies to ampicillin using disk diffusion. Percentage of ciprofloxacin resistance varied from 0%-95%, while ampicillin ranged from 0 to 100%. In this review, there was a study by Ahmed et al.[29] which showed the ciprofloxacin susceptibility data as sensitive, intermediate, and resistant isolates. Intermediate susceptible *Salmonella* Typhi were classified as resistant isolates.

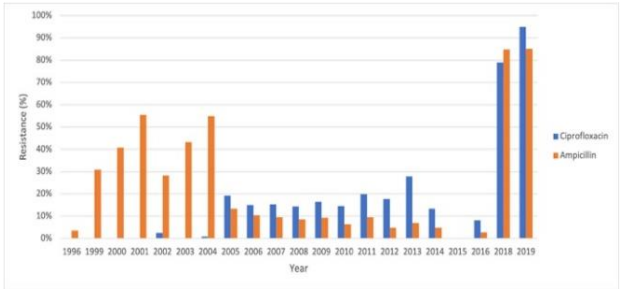

Figure 2. Percentage of *Salmonella* Typhi Isolates Rsistant to Ciprofloxacin and Ampicillin in Asia from 1996 to 2019.

Figure 2 represents the percentage of *Salmonella* Typhi isolates resistant to ciprofloxacin and ampicillin in each year that were tested with disk diffusion method.

Number of resistant *Salmonella* Typhi isolates reported from 1996-2019 were combined (Figure 2). These data were extracted from India, Nepal, Bangladesh, Pakistan, Iraq, and Indonesia. However, there was no data found that reported results from the year 1997, 1998, and 2017.

In a five year period from 1996 until 2001, there was no ciprofloxacin-resistant *Salmonella* Typhi found. However, there were 3.5%, 30.9%, 40.7%, and 55.5% *Salmonella* Typhi resistant to ampicillin. The number of *Salmonella* Typhi resistant to ciprofloxacin contrasted with ampicillin that increased in those years.

In 2002 to 2003, *Salmonella* Typhi remained relatively sensitive to ciprofloxacin, while highly resistant toward ampicillin. Studies from South Asia in 2004-2005 reported a huge turning point where *Salmonella* Typhi resistant to ciprofloxacin increased from 0.8% to 19.3%, while ampicillin decreased from 54.9% to 13.3%. In the year 2005 until 2014, the number of

Salmonella Typhi resistant to ciprofloxacin always remained higher than the one resistant to ampicillin. Resistance toward ciprofloxacin varied from 13.4% to 27.8% while ampicillin ranged from 4.8% to 13.3%. *Salmonella* Typhi resistant to ampicillin reached 4.8% in 2012, whereas *Salmonella* Typhi resistant to ciprofloxacin increased reaching 27.8% in 2013.

In 2015, a report by Lugito et al.[32] found that no *Salmonella* Typhi was resistant to ciprofloxacin and ampicillin in Indonesia. Furthermore, other studies from 2016 to 2019 reported percentage of *Salmonella* Typhi resistant to ciprofloxacin and ampicillin increased simultaneously; however, it must be noted that, in 2016 and 2019, only one study in each year reported the resistant isolates.

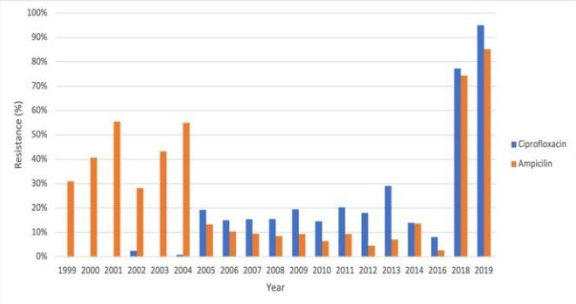

Figure 3. Percentage of *Salmonella* Typhi isolates that are resistant to Ciprofloxacin and Ampicillin in South Asia from 1999 to 2019.

The resistance trend to ciprofloxacin and ampicillin of *Salmonella* Typhi in South Asia countries included from India, Pakistan, Bangladesh, and Nepal is shown in Figure 3. From 1999 until 2004, *Salmonella* Typhi appeared to be relatively susceptible toward ciprofloxacin, while resistance toward ampicillin was quite high ranging from 28.2% to 55.5%. The breaking point was seen in 2005, where the percentage of *Salmonella* Typhi that was resistant to ciprofloxacin increased while ampicillin decreased. The percentage of *Salmonella* Typhi isolates resistant to ciprofloxacin continued to exceed isolates resistant to ampicillin until 2019. From 2005 until 2013, *Salmonella* Typhi resistant to ciprofloxacin remained high, on the other hand, resistance to ampicillin gradually decreased. In 2014, the percentage of *Salmonella* Typhi resistant to ciprofloxacin and ampicillin were relatively similar, with resistance toward ciprofloxacin slightly higher than ampicillin. In 2016, *Salmonella* Typhi resistant to ampicillin fell to 2.7%. From 2018 until 2019, there was a further increase in ampicillin-resistant isolates with both year results reported by Shah et al.[30] and Saeed et al.[31] from Pakistan. This showed that Pakistan had a high percentage of *Salmonella* Typhi resistant to ampicillin in 2018 and 2019.

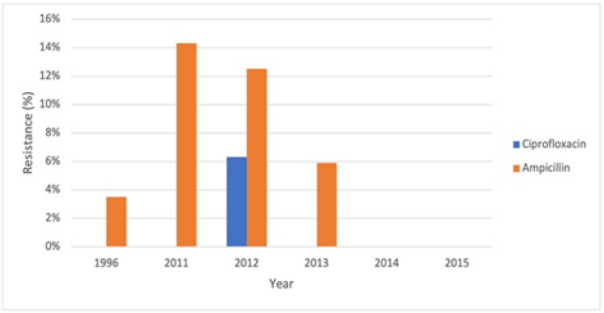

Figure 4. Percentage of *Salmonella* Typhi Isolates that Are Resistant to Ciprofloxacin and Ampicillin in Southeast Asia from 1996-2015.

Salmonella Typhi resistant to ciprofloxacin and ampicillin in South-East Asia is shown in Figure 4. Since 1996, it showed that *Salmonella* Typhi was resistant to ampicillin while there was no isolate that is resistant to ciprofloxacin until 2012. Percentage of *Salmonella* Typhi resistant to ciprofloxacin seemed to be low. This study aligns with studies from Moehario et al.[35] which reported there was no *Salmonella* Typhi isolates that were resistant to ciprofloxacin in 2002-2010, which showed low ciprofloxacin resistance in Indonesia. Percentage of *Salmonella* Typhi that was resistant to ampicillin increased from 1996 to 2011 and decreased each year reaching 0% in

2014 and 2015. A study from Thailand conducted by Techasaensiri et al.[36] reported similar results from 1998 through 2007, *Salmonella* Typhi and *Salmonella* Paratyphi resistant to ampicillin remained below 40% and decreased as of 2007.

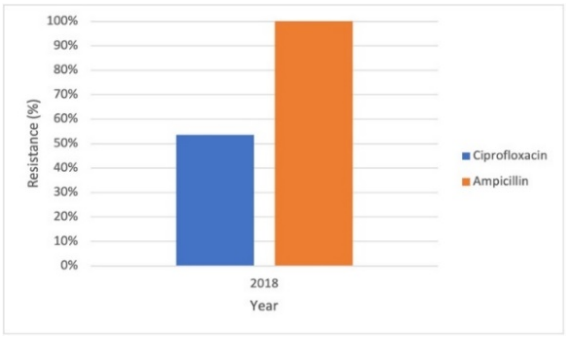

Figure 5. Percentage of *Salmonella* Typhi isolates resistant to Ciprofloxacin and Ampicillin in East Asia.

Figure 5 represents *Salmonella* Typhi isolates resistant to ciprofloxacin and ampicillin that were tested with disk diffusion method and obtained from studies originating from Iraq.

The resistance trend in East Asia only consists of one study from Iraq (Figure 5). This study reported a high level of *Salmonella* Typhi resistance in 2018, which was 53.6% resistant to ciprofloxacin and 100% resistant to ampicillin. This showed similar with results found in Nepal and Pakistan in 2018. Khadka et al.[25] reported 43.5% *Salmonella* Typhi resistant to ciprofloxacin in Nepal. Saeed et al.[31] reported 90.2% reported 90.2% isolates resistant to ciprofloxacin and 74.4% resistant to ciprofloxacin in Pakistan.

Despite all data that have been analyzed, this study still could not represent the whole of Asia, since the studies collected thus far originated from India, Pakistan, Bangladesh, Nepal are South Asia countries, Iraq an East Asia country, and Indonesia a Southeast Asia country, furthermore, limited number of samples were tested in several included studies, and fewer studies found *Salmonella* Typhi resistant to ampicillin more reported amoxicillin instead.

STRENGTH AND LIMITATION

The strength of this study was using PRISMA method as the foundation for doing this study. Critical appraisal was also done for all the studies included. On the other hand, the limitation of this study was we could not include some of the studies which were not in English or Indonesian language.

CONCLUSION

In Asia, there was an increase in ciprofloxacin-resistant *Salmonella* Typhi from 1996 until 2019, while, on the contrary, ampicillin-resistant *Salmonella* Typhi decreased from 1996 until 2015. In 2016 until 2019, contrasting evidence was found in East Asia and South Asia where resistance toward ampicillin rose.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Writer, literature searcher, collecting data from literature: FT. Conceptor and supervision: LHM and IMN. Review and supervision: JN, AMRLT and SS.

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Case Report

Purple Urine Bag Syndrome: a Rare Manifestation of Urinary Tract Infection

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ABSTRACT

Purple urine bag syndrome (PUBS) is rare manifestation of urinary tract infection (UTI). Epidemiological study showed the prevalence of purple urine bag syndrome about 8.3%-16.7% worldwide. There are some factors which lead to the disease including female, long-term urinary catheter, bedridden or immobile for long time, constipation, and urinary tract infection. The mechanism of this condition involves the tryptophan in intestine that is degraded into indole. In the liver, indole is conjugated into indoxyl sulphate. This conjugate product then is excreted into urine by the kidney. In the infected urinary tract, some gram-negative bacteria produce enzymes called sulphatase and phosphatase. It converts the conjugated product, indoxyl sulphate into pigments, red indirubin and blue indigo. The two pigments-combination produces purple pigment which appears in urine. We present a-61-year-old female who has history of cerebrovascular accident who came to our emergency room with purple urine over the previous seven days.

Keywords: : Purple Urine Bag Syndrome, Urinary Tract Infection, Chronic Urinary Catheterization, Beta Lactamase, and Escherichia coli.

Highlights: an adequate antibiotic treatment of UTI bring the clinical improvement of purple urine bag syndrome. Urine culture may give the benefit in choosing appropriate antibiotic.

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INTRODUCTION

Purple Urine Bag Syndrome (PUBS) is a rare manifestation Urinary Tract Infection (UTI) and first reported in 1978[1]. The unusual urine color can be distressing for the patient and families. Actually, the first case of purple urine bag syndrome was reported in 1812; this phenomenon happened to King George III.[2] Epidemiological study reported prevalence of PUBS 8.3%-16.7%.[3,4] Literature review by Yang et al.[5] collecting data from October 1980 to August 2016 showed 116 case reports with PUBS.[5,6] Female, elderly patient, constipation and chronically debilitated are considered to be the risk factor of PUBS.[3,7] This condition has been considered as benign condition and appropriate antibiotic remains to be suggested for its treatment.[5] We report a-61-year-old female with CVA pneumonia and PUBS as complication of UTI et causa prolong catheterization with *Escherichia coli* ESBL +.

CASE REPORT

A-61-year-old female with a background of cerebrovascular accident and chronic hypertension presented to emergency room with purple urine over the previous seven days (Figure 1). Due to neurogenic bladder, she had foley catheterization for one year which was changed every two weeks. For a few days, she lost appetite and did not drink enough water. There was no history of fever but she complained of pain on the tip of urethra. She was also suffering from melena and pneumonia. Her recent medication: adalat oros, candesartan, clopidogrel and citicoline.

The vital signs were stable while physical examination revealed pale conjunctiva, bilateral rhonchi, ascites and pitting edema in upper and lower extremities. Thorax photo showed pneumonia with pleural effusion. Laboratory test showed anemia (Hb 6.3, normal range 11.7-15.5), hypoalbuminemia (1.2, normal range 3.4-4.8), slight increase of creatinine serum (1.66, normal range 0.45-0.75) and BUN (40, normal range 4.6-23). Her urinalysis at the emergency room showed pH 8, Protein +3, leucocyte esterase 500, Leucocyte 25-30/HPF, erythrocyte 2-4/HPF and bacteriuria +. While urine sample was sent for culture and antibiotic sensitivity test, patient was treated by ceftriaxone 2x1 gram. The foley catheter was also changed. She received transfusion of PRC and albumin to treat anemia hypoalbuminemia.

After three days of admission, urine returned to yellow, but the symptom of UTI was not completely improved. Second urinalysis was conducted and it showed pH 7.5, albumin +2, leucocyte esterase 500, blood +1, leucocyte 10-15/HPF, erythrocyte 15-20/HPF, uric amorphous +, cast + and bacteriuria ++. Urine culture revealed significant growth of *Escherichia coli* ESBL+ (>10^5 cfu/ml) which was sensitive to amikacin, doripenem, ertapenem, meropenem, nitrofurantoin, minocycline and tetracycline. The patient was then given intravenous meropenem 3x1 gram for five days. After antibiotic was changed, the symptoms of UTI improved. The patient was discharged after 14 days of treatment.

Figure 1. Purple Urine in Urine Bag and Tubbing.

DISCUSSION

Purple Urine Bag Syndrome (PUBS) is an uncommon manifestation of UTI with prevalence 9.8% in patients with long term urinary catheter use.[8] The mechanism of PUBS involves a sequence reaction of dietary digestion and absorption of tryptophan in the intestine.[6,9] The tryptophan in the intestine is degraded by the bacteria and produces indole.[5,10] Indole transported to liver by hepatic circulation and hepatic enzyme converts indole into conjugate indoxyl sulphate. [1,5,9] The indoxyl sulphate is secreted into the urine by kidney.[5] The sulphatase and phosphatase produced by certain gram-negative bacteria in the urinary tract converts the indoxyl sulphate into blue indigo pigment and red indirubin pigment through the oxidation process.[1,5] The pigments combine causing purple staining of the urine. Not all the same species of bacteria produce sulphatase and phosphatase, but the bacteriuria is always present in all patients with PUBS even those without clinical symptoms of UTI[5]. Hepatic enzymes, bacterial urine oxidation, and the combination of indigo, a blue pigment, and indirubin, a red pigment, are the causes of the purplish discolorations of PUBS. The mechanism of purple urine bag syndrome us shown in Figure 2 below.

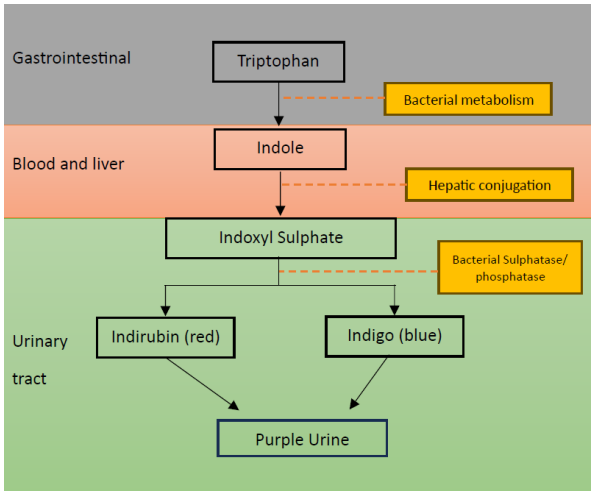

Figure 2. Mechanism of PUBS.

Based on the pathogenesis of PUBS, UTI is the factor developing the disease.[10] It is because the bacteria contribute to produce enzyme degrading indoxyl sulphate into the pigment so that the urine became purple.[5] All of the factors for UTI are also indirectly become risk factors for PUBS. Female has shorter urethra than male so that they are more likely to develop UTI.[4] Some of studies showed female obviously associated with PUBS.[5,10,11] The picture of PUBS in an old female patient are shown in Figure 3. In this presented case, the patient was an old debilitated female who complained pain on the tip of urethra which was a common symptom of UTI. She was on urine catheterization for a long time which is also the risk factor of PUBS. The condition of bedridden in this patient also has role in developing the disease. The bedridden condition is prone to reduce gut motility which is found relating to PUBS.[4] In this condition of patient, urine catheter cannot be removed and it is important to reduce the risk of infection by improve the hygiene.[12]

Figure 3. The PUBS in an Old Female Patient with Long-Term Urinary Catheter.[10]

The bacteria that are most commonly associated with PUBS are

Providencia stuartti and *rettgeri*, *Proteus mirabilis*, *Pseudomonas auruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Morganella*, and *citrobacter* species, Enterococci, and Group B Streptococci [2,12] The resistance organisms are infrequently reported.[11] The bacterium found in this patient was *E. coli.* The species of bacteria are the same with previous study, but in this case the bacteria produce extended-spectrum-beta-lactamase enzyme. The prevalence of extended spectrum beta-lactamase is high in Southeast Asia, Africa and Central America.[13] *E. coli* and *Klebsiella pneumoniae* are frequently organisms that produce its enzyme to became resistant to beta-lactam antibiotics including cephalosporin-third generation and penicillin, which are most commonly used due to broad spectrum activity and less toxicity.[14,15]

Urine culture and antibiotic sensitivity test have a significant role for the treatment in this presented case. Urine culture shows type of pathogen and sensitivity test helps the physician to consider antibiotic treatment especially when there is no improvement after empirical antibiotic. In this patient, the symptom of UTI did not improve with ceftriaxone as an empirical antibiotic. After she got cultured guide antibiotic, the symptom of UTI was diminished.

Alkaline urine is also considered as an associated factor because indoxyl turns into indigo and indirubin in alkaline condition.[4] Some cases, reported the patients with PUBS with alkaline urine.[1,6,8] The urinalysis of this patient revealed pH > 7. However, it had ever been reported PUBS in acidic urine and it has shown us that the pH is not a causative factor, but an associated factor.[4]

PVC plastic catheter is also reported to contribute in developing the disease[1,8]. PUBS is more frequent in patients with PVC urine bag than non-PVC urine bag. The interactions between the plastic urine catheter bag, the pigment produced by the bacteria and high bacterial load have a significant role in the disease[1].

There are many factors which contribute to develop the disease. Despite this, it is a benign ; purple urine bag syndrome is an indicate recurrent UTI due to improper hygiene[8]. Since there is no specific guideline for the disease, the management of UTI including antibiotic therapy is considered to be the important thing in PUBS treatment. Besides, good hygiene, changing the catheter regularly, considering non plastic catheter bag, treating underlying medical condition, and control of modified risk factors also bring about its resolution.

STRENGTH AND LIMITATION

The strength of this study is this was a rare case so it can give insight to manage a similar condition. The limitation of this study is the case presented is only a single case, so it is limited.

CONCLUSIONS

PUBS is rare manifestation of UTI and considered as a benign condition. There are many factors which contribute in developing the disease. Appropriate antibiotic treatment, good hygiene, good catheter care, treating underlying medical condition which precipitate to the disease and control of modified risk factors are the key in PUBS treatment.

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ETHICAL CLEARANCE

This research was approved by the Health Reseach Ethics Committee of

UOBK RSUD Syarifah Ambami Rato Ebu Bangkalan with number: 0047/KEPK/XII/2023.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

IDT involved in conceptualization, collecting data, writing manuscript. RF involved in conceptualization, data review, and supervision. CBK involved in conceptualization, data review, and supervision.

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Indonesian Journal of Tropical and Infectious Disease

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