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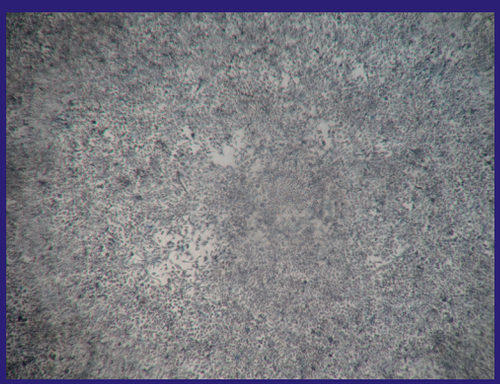

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Research Article

Correlation Analysis between Ratio of C-Reactive Protein/Albumin and Severity of Dengue Hemorrhagic Fever in Children

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is a dengue infection which can cause shock and leads to mortality. Hypoalbuminemia is a marker of plasma leakage in DHF and correlated with severity of inflammatory response triggered by infection, including DHF. C-Reactive Protein (CRP) is a proinflammatory marker that also increases in DHF. This study aims to determine a correlation of CRP/albumin ratio with severity of DHF. Cross sectional study on pediatric patients diagnosed as DHF at Saiful Anwar Malang Hospital was done in July-December 2016. CRP levels were examined using immunoturbidimetry method, while albumin was examined by using Bromocresol Green (BCG) method. Correlation of CRP/albumin ratio with DHF severity was analyzed by using Pearson correlation test.The result showed that there were significant differences in CRP levels and CRP/albumin ratios in the Dengue Shock Syndrome (DSS) and non-DSS group ($p = 0.002$, $p = 0.001$, $\alpha < 0.05$). There was no significant difference in albumin level in the same group ($p = 0.207$, $\alpha < 0.05$). Positive correlation found in CRP and CRP/albumin ratio ($r = 0.46$, $r = 0.49$, $\alpha < 0.01$). On the contrary the negative correlation was found in albumin ($r = -0.21$, $\alpha < 0.01$). This is presumably because albumin is an acute phase protein which will decrease along with the severity of infection. In contrast, CRP will increase during the critical phase of infection. It can be concluded that the CRP/albumin ratio was positively correlated with DHF severity, as well as CRP levels, but not positively correlated with albumin.

Keywords: *Dengue Hemorrhagic Fever; Dengue Shock Syndrome; Severity; CRP/Albumin Ratio; CRP*

ABSTRAK

Demam Berdarah Dengue (DBD) merupakan Infeksi Virus Dengue (IVD) yang dapat menyebabkan syok dan berakhir dengan kematian. Hipoalbuminemia merupakan salah satu penanda kebocoran plasma pada DBD sekaligus berkorelasi dengan intensitas respon inflamasi yang dipicu oleh infeksi, termasuk DBD. CRP juga merupakan marker inflamasi yang juga meningkat pada DBD. Penelitian ini bertujuan untuk mengetahui apakah rasio CRP/Albumin berkorelasi dengan keparahan pada DBD. Penelitian cross sectional pada pasien anak dengan diagnosis DBD dilakukan di RS Saiful Anwar Malang pada Juli-Desember 2016. Kadar CRP diperiksa menggunakan metode imunoturbidimetri, sedangkan kadar Albumin diperiksa menggunakan metode Brom Cresol Green (BCG). Korelasi Rasio CRP/albumin dengan keparahan DBD dianalisis menggunakan uji korelasi Pearson. Hasil penelitian menunjukkan terdapat perbedaan kadar CRP dan Rasio CRP/Albumin yang bermakna pada kelompok Dengue Syok Syndrome (DSS) dan non DSS ($p= 0.002$, $p= 0.001$, $\alpha < 0.05$). Tidak didapatkan perbedaan kadar albumin yang bermakna pada kelompok yang sama ($p=0.207$, $\alpha<0.05$). Korelasi positif sedang ditunjukkan oleh CRP dan Rasio CRP/Albumin ($r=0.46$, $r=0.49$, $\alpha<0.01$). Sebaliknya korelasi negatif didapatkan pada albumin ($r = -0.21$, $\alpha<0.01$). Hal ini diduga karena albumin merupakan protein fase akut yang akan turun seiring dengan beratnya infeksi. Sebaliknya, CRP akan meningkat selama fase kritis. Dapat disimpulkan

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bahwa rasio CRP/Albumin berkorelasi positif sedang dengan keparahan DBD, demikian pula dengan kadar CRP, namun tidak berkorelasi positif dengan albumin.

Kata kunci: *Demam berdarah Dengue; Sindrom Syok Dengue; Keparahan; rasio CRP/ albumin; CRP*

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INTRODUCTION

Dengue virus (DENV; family of Flaviviridae, genus Flavivirus) is transmitted by Aedes aegypti mosquitoes and can cause relatively mild Dengue fever (Dengue Fever-DF); or more severe form of dengue (Dengue Hemorrhagic Fever-DHF).[1, 2] Severe organ damage does not occur much but if it occurs, it can cause mortality because it is slowly detected. Severe organ damage is one of the leading causes of mortality besides shock.[3-6] Therefore, it needs a marker which can predict organ damage.

Considering the clinical manifestations of dengue infection which vary from mild to severe and the result is difficult to predict, a predictor biomarker is needed to act as an early warning sign.[7-12] Suwarto in his study in 2016 has developed dengue scores to predict pleural effusion and/or ascites in adults in dengue infection. The study showed that hemoconcentration was ≥15.1%, albumin concentration in the critical phase was ≤3.49g/dL, platelet count was ≤49,500/μL, and high AST ratio was ≥2.5 had sensitivity and specificity above 60%.[4, 5, 13]

Another reliable biomarker when critical is C-reactive protein (CRP). CRP is an acute phase protein produced by hepatocytes, especially under IL-6 control which has been proven as a sensitive prognostic indicator of inflammation.[14, 15] Ranzani in his study (2013) on CRP shows that CRP can be used as a diagnostic tool for sepsis and for therapeutic monitoring. The measurement of CRP level can also help clinicians in making decisions whether patients need an ICU or not.[16] Grander (2010) has shown that CRP level correlates with the level of inflammation at the beginning of the diseases course. Although some studies have shown that CRP level when exiting from ICU can be a reliable marker in monitoring but no studies have focused on dengue patients.[17]

Not only for CRP, but serum albumin can also be an important short- and long-term marker in determining prognosis. Serum albumin is a negative acute phase protein, thus the level of hypoalbuminemia in critically ill patients correlates with the intensity of the inflammatory response triggered by infection. Therefore, CRP and serum albumin level must be inversely proportional during the critical phase. The use of CRP and albumin ratio will provide a variable which is able to combine information provided by CRP and albumin. Therefore, it can be an index which has a positive correlation with infection, a higher ratio indicates a higher inflammatory status. CRP/albumin ratio has been widely investigated in cases of malignancy. One of Liu's studies in 2015 showed that AUC was 0.625, $p < 0.001$, for the role of the CRP/albumin ratio as an independent prognostic marker in the preoperative of gastric cancer circumstance. The study underlines that the CRP/albumin ratio not only reflects inflammation but also the nutritional status of cancer patients.[16]

Based on the research background, infection is one of the strongest triggers for inflammation, we hypothesized that CRP and albumin level would be important markers of dengue severity. In addition, we also investigated whether the combination of information from CRP and albumin through the CRP/albumin ratio would improve the quality of the prognostic marker of dengue severity when compared to CRP or albumin only.

MATERIALS AND METHODS

This study was retrospective and was conducted on all pediatric patients with a diagnosis of DHF

who were treated in the Pediatric Ward of Saiful Anwar Malang Hospital during July-December 2016. The data were obtained from medical records then the data were carried out descriptive analysis. The population of the study subjects was divided into two groups of dengue severity: dengue shock (DHF grade 3,4) and dengue non-shock (DHF grade1,2).

The inclusion criteria in this study were DHF pediatric patients who were hospitalized with positive NS-1 laboratory results and/or IgM anti dengue immunoserology test and/or positive IgG and examined serum albumin and CRP on the same day during treatment. Another inclusion criteria is the patients who diagnosed DHF and were <18 years old. The diagnosis of DHF was based on WHO 2011 criteria. The patients who were willing to be included in this study signed informed consents. While the exclusion criteria were the subjects who suffered from another infection which could produce false positives on immunoserology dengue examination (e.g., malaria, typhoid fever). To provide sufficient power in cross sectional study, at least 32 children were needed according to the sample size formula:

$$N = \frac{Z\alpha + Z\beta}{0.5 \ln [(1+r)/(1-r)]}^2 + 3$$

$$N = \frac{1.64 + 1.28}{0.5 \ln [(1+0.5)/(1-0.5)]}^2 + 3$$

= 32 sampel

Patients who became the sample were patients who came to the Child Polyclinic and Emergency Room of Dr. Saiful Anwar Malang General Hospital, fulfilled the inclusion and exclusion criteria for clinical and laboratory examinations. The sample's inclusion and exclusion criteria were determined by history, physical examination, completely blood laboratory examination, clinical chemistry, and immunoserology. Patients' serum were collected in laboratory and then stored at -80°C. When samples collection is completed, all serum were tested CRP and albumin.

This study was approved by the local medical ethical committee with ethical clearance number 400/196/K/3/302/2017.

The data analysis consists of several tests. Shapiro-Wilk test was used to see the data normality. Mann Whitney T-test was used to see the mean differences in the two groups. Pearson test was used to determine the relationship of CRP, albumin, and CRP/albumin ratio with the severity degree of dengue infection/prognosis. ROC curve was used to see the performance of CRP single marker, albumin, and combined marker of CRP/albumin ratio.

RESULTS

Characteristics of Subjects

Thirty-nine pediatric patients infected with dengue virus were included in this study consist of 17 samples dengue non-shock and 22 samples dengue shock (Table I, II, III). All patients were tested albumin and CRP.

Table I. Characteristic of Subject Based on Age

Patients' age	Prognosis		Total
	Dengue without shock	Dengue with shock	
0-1 year	5	5	10
1-5 years old	5	6	11
5-10 years	5	8	13
11-15 years old	2	3	5
15-18 years old	-	-	-
Total	17	22	39

Table II. Characteristic of Subject Based on Gender

Gender	Prognosis		Total
	Dengue without shock	Dengue with shock	
Male	11	5	16
Female	6	17	23
Total	17	22	39

Table III. Characteristic of Subject Based on Nutritional Status

Nutritional Status (*Z*-score BB/TB)	Prognosis		Total
	Dengue without shock	Dengue with shock	
Very Thin (<-3SD)	1	0	1
Thin (-3SD to <-2SD)	0	3	3
Normal -2 SD to 2 SD	15	17	32
Fat > 2 SD	1	2	3
Total	17	22	39

Data Analysis

The normality test showed the distribution of abnormal data for age, gender, and nutritional status. The results of the post-transformation normality test data also showed the data distribution which was not normal so that the different test analysis used Mann-Whitney. The results of different test showed that there were significant differences in gender data in the shock and non-shock groups (Table IV).

Table IV. Difference Tests Based on Age, Gender, Nutritional Status in Shock and Non-Shock Groups (95% Confidence Interval)

Different Test	Normality test	p
Based on Age (*Mann Whitney*)	0.004	0.136
Based on Gender (*Mann Whitney*)	0,000	0.009
Based on Nutritional Status (Mann *Whitney*)	0,000	0.470
CRP (T-*Test*)	0.164	0.002
Albumin (t-*Test*)	0.653	0.207
CRP/Albumin Ratio (t-*Test*)	0.149	0.001

Based on the normality test it was obtained the distribution of normal data for albumin (0.653), but there is an abnormal distribution of data (<0.05) for CRP and CRP/albumin level data, so that transformation needed to be done. The Shapiro-Wilk Normality Test showed the distribution of post-transformation normal data which 0.164 for CRP and 0.149 for CRP/albumin Ratio were so that data analysis could be continued using parametric tests. The results of different marker tests showed only CRP level which showed significant difference in the shock and non-shock groups (Table IV).

The correlation tests showed positive correlations for CRP and CRP/Albumin level, but there was a negative correlation for albumin level (Table V).

Table V. Pearson Correlation Test, with 99% Confidence Interval

Pearson Correlation Tests	r	p
Dengue Group and CRP Level	0.46	0.003
Dengue Group and Albumin Level	-0.21	0.199
Dengue Group and CRP/Albumin Ratio	0.49	0.002

Furthermore, the data analysis was performed with receiver operating characteristic (ROC) curve, AUC (Area Under the Curve) to see the performance of markers (Table VI, Table VII, Table VIII, Table IX, and Figure 1).

Table VI. Area Under the Curve

Test variable	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower limit	Upper limit
CRP	0.218	0.075	0.003	0.071	0.365
Albumin	0.616	0.092	0.218	0.435	0.797
CRP / Albumin Ratio	0.203	0.072	0.002	0.061	0.345

Table VII. CRP Prognostic Test

	Shock	Non-Shock	Total
> 0.5 mg/dL	15	5	20
≤ 0.5 mg/dL	7	12	19
Total:	22	17	39
Sensitivity:	68.18%		
Specificity:	70.59%		
PPV (Positive Predictive Value):	75%		
NPV (Negative Predictive Value):	63.16%		
RR (Relative Risk):	2.02		

Table VIII. Albumin Prognostic Test

	Shock	Non-Shock	total
≤ 2.7 mg/dL	5	0	5
> 2.7 mg/dL	17	17	34
Total:	22	17	39
Sensitivity:		22.73%	
Specificity:		100%	
PPV (Positive Predictive Value):		100%	
NPV (Negative Predictive Value):		50%	
RR (Relative Risk):		2	

Table IX. Prognostic Test for CRP/Albumin Ratio

	Shock	Non-Shock	total
≥ 0.2	14	5	19
<0.2	8	12	20
Total:	22	17	39
Sensitivity:		63.64%	
Specificity:		70.59%	
PPV (Positive Predictive Value):		73.68%	
NPV (Negative Predictive Value):		60%	
RR (Relative Risk):		1.83	

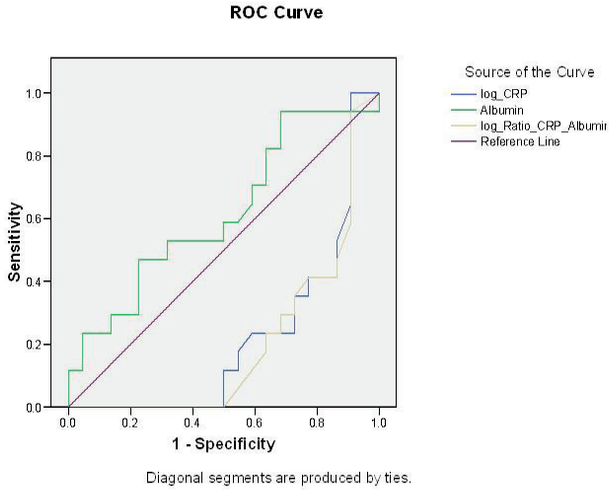

Figure 1. ROC Curve of Prognostic Test of CRP, Albumin, CRP/Albumin Ratio against Severity Degree of Dengue Infection

DISCUSSION

In the baseline data, there were significant differences in different tests based on gender. In this case the female patients were significantly (n=17) more than male patients (n=5) in the dengue group with shock. This is not the same as Lovera's study in 2016 which found that there was no gender preference in severe dengue manifestation.[18] Also this is not the same as Anker's study in 2011 in which his study looked at the incidence of dengue infection in children in Asia, the data showed that the number of male cases was significant in the age ≥ 15 years group. This difference based on gender was indeed not supported by specific pathophysiological mechanisms. The difference possibility related to gender in dengue fever was due to difference in exposure in the adolescent age group. The results of this study in Asia were different from those in South America, where there was a similar proportion of male and female patients in dengue fever cases or conversely the proportion of female cases were greater. The reason for this difference of incidence based on gender needs to be explored further.[19]

Furthermore, there were no significant difference based on age and nutritional status. Although most of the samples were form age 5 – 10 year old group which is similar to Lam et al. study.[20] Our nutritional status was analyzed by Z-score: weight/height (BB/TB). Based on previous studies, moderate/severe malnutrition was associated with a significant reduction in cell-mediated immunity, as indicated by a reduction in the number of $CD4^+$T cells, and a decrease in the $CD4^+/CD8^+$ ratio. There was also a decrease in secretory IgA antibody production and various component supplements (C3, C4, and factor B) and decreased phagocytosis. The production of certain cytokines such as IL-2 and TNF also decreases.[21] The study done by Kalayanarooj in 2005 study concluded that malnourished children had a lower risk of dengue infection, but if they were infected with dengue they had a high risk of DSS. Obese children had a higher risk of contracting dengue fever with a more unusual presentation; encephalopathy, related infections and complications of excess fluid.[22] Widiyati's study mentioned that obesity is not a risk factor for children with dengue infection to get DSS.[23]

Furthermore, in this study there was no significant difference in the different albumin tests (unpaired T test) 0.207, whereas for CRP

and CRP/Albumin ratios there were significant differences, 0.002, 0.001 respectively.

Albumin synthesis experienced significant changes in the critical phase. As acute responses to trauma, inflammation, and sepsis, it would improve the transcription process of acute phase proteins such as CRP and would reduce transcription of albumin mRNA and albumin synthesis. Both IL-6 and TNF-α could reduce gene transcription. Based on Liao's study in 1986, the induction of inflammation in mice was done to see changes in albumin levels. The study showed the lowest albumin levels were obtained at 36 hours and then rose again. A sustained inflammatory response in critical illness could result in a long barrier to albumin synthesis as well.[24]

The Fairclough's study in 2009 mentions low albumin levels were most often associated with chronic diseases, also often associated with malnutrition.[25] Napoleon-Tatura et al. found that low albumin levels can help predict shock in pediatric dengue.[26] This study included an acute case study so that not all critical patients showed a decrease in serum albumin during treatment. Fever days when samples taken, and nutritional status also varied so that the results obtained did not match the theory.

Based on the Pearson correlation test, the same correlation was found between the relationship between CRP (r = 0.46) and CRP/Albumin Ratio with the friction of Dengue infection (r = 0.49). This means there was a weak relationship. In accordance with the Liao's in 1986 that the day when sampling taken was very influential, and in the study indeed the data of the sampling taken was not homogeneous.[24]

While the correlation of the relationship between albumin and severity of dengue infection was r = -0.21. It is according to the theory that albumin is an acute phase protein that will decrease along with the severity of infection/inflammation. But in this study a weak correlation was also found for albumin. Based on Fairclough's study in 2009, nutritional status was very influential on albumin levels, while the nutritional status in this study was not homogeneous either.[25]

Based on the prognostic test, the results were almost the same, both the sensitivity and specificity between CRP and CRP/albumin ratio were 68% and 70% respectively for CRP performance; and 63% and 70% for CRP/albumin performance ratio. CRP was at the cut off > 0.5mg/dL and the CRP/albumin ratio was at the cut off > 0.2. The CRP and CRP/albumin ratio had similar AUC (0.218; 0.203) with $p < 0.05$, whereas AUC albumin was not significant.

Menon's study in 2005 using cut-off CRP > 3 mg/L could be an independent marker of mortality risk factor in cardiovascular disease. The previous study on CRP/Albumin ratio used quite varied cut-offs.[27] Wei's study in 2015 used cut-off > 0.095 which was associated with the size of esophageal tumor (squamous cell carcinoma).[28] While Xie's study in 2011 used cut-off > 0.42 this was associated with mortality in AKI patients.[29] Liu et al found that patients with pancreatic cancer that have CRP/albumin ratio ≥ 0.18 have worse prognosis than those with CRP/albumin ratio < 0.18.[30] Based on this study both CRP and CRP/albumin ratio were as good at predicting the severity of dengue infection.

Whereas serum albumin of cut-off < 2.7mg/dL showed a low sensitivity of 22%, so it could not be used as a single marker of initial screening predictor of dengue infection severity. However, for the cut off, serum albumin had a specificity of 100% which could specifically direct the severity that occurs in patients with dengue infection.

Based on this study the best relative risk (RR) was in CRP (RR = 2.02) and albumin markers (RR = 2), in which the values were almost the same, followed by CRP/albumin ratio (RR =1.83). However, all markers had a value of RR >1 so that they could be used to see the probability of dengue prognosis.

From ROC curve, only albumin that has good performance with AUC 0.616 and $p = 0.218$, while CRP and CRP/albumin ratio has AUC = 0.218 and AUC = 0.203 with $p = 0.003$ and $p = 0.002$ respectively (Figure 1).

This study had limitations that must be considered. This study was conducted on patients without comorbidity, the sampling done on the varied fever days, the nutritional status was not

homogeneous. It was better if it was tested in populations with comorbidities, especially in people with comorbidities having potential to affect the levels/concentrations of predictive variables, such as kidney disease and liver disease. It was also best to do homogenization of sampling days and nutritional status.

CONCLUSION

Both CRP and CRP/albumin ratio are independent prognostic markers of the severity of dengue infection. The use of this ratio is easy, inexpensive, and has sufficient availability, so it is very helpful for clinicians to identify high-risk dengue patients. Single serum albumin cannot be used as a screening marker for the severity of dengue infection.

Further studies to predict the severity of dengue infection should include a population with comorbid kidney disease and liver disease, and collect more samples, including the participation of adult patients. Hopefully the best predictor markers can be found.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Original Article

Analysis of HIV/AIDS Health Problems in Pacitan District East Java 2020

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ABSTRACT

The implementation of health problem analysis is carried out to increase the effectiveness and efficiency of solving health problems through the selection of health problems that become priority problems in a region. The purpose of this study was to analyze the problem and determine the priority of health problems in the work area of the Pacitan District Health Office, East Java Province. This research is a descriptive observational study conducted at the Pacitan District Health Office in January 2020. The type of data used is secondary data obtained from the 2016-2019 Pacitan District Health Profile and primary data obtained through interviews with related parties, namely the head of the field. , section head and program holder. Prioritization of health problems is carried out using the USG method based on the criteria of Urgency, Seriousness, Growth and finding the root of the problem using the fishbone diagram method. The increase in HIV/AIDS cases with an USG score of 128 has become a top priority health problem in Pacitan District. An increase over the last 4 years with the highest number of cases in 2019, which was 39 cases. The fishbine diagram shows the root of the HIV/AIDS problem, namely the lack of public knowledge about HIV/AIDS, the lack of public knowledge about HIV/AIDS, the lack of awareness of people at risk for conducting an HIV test, this makes the community less aware of information about HIV/AIDS, causing public stigma. which results in people being closed / unwilling to check themselves at the puskesmas or hospital. The increase in HIV/AIDS cases is one of the problems in Pacitan district. To reduce the incidence, health workers need to optimize the dissemination of information about HIV/AIDS, especially risk factors, causes, prevention, symptoms and treatment. Increase the understanding of health workers and public awareness in conducting early detection.

Keywords: *HIV/AIDS; Urgency; Seriousness; Growth.*

ABSTRAK

Pelaksanaan analisis masalah kesehatan dilakukan untuk meningkatkan efektivitas dan efisiensi penyelesaian masalah kesehatan melalui pemilihan masalah kesehatan yang menjadi prioritas masalah di suatu wilayah. Tujuan dari penelitian ini adalah untuk melakukan analisis masalah dan menentukan prioritas masalah kesehatan yang ada di Wilayah kerja Dinas Kesehatan Kabupaten Pacitan Provinsi Jawa Timur. Penelitian ini merupakan penelitian deskriptif obervational yang dilakukan di Dinas Kesehatan Kabupaten Pacitan pada bulan Januari tahun 2020. Jenis data yang digunakan yaitu data sekunder yang diperoleh pada Profil Kesehatan Kabupaten Pacitan tahun 2016-2019 dan data primer yang diperoleh melalui wawancara dengan pihak terkait yakni kepala bidang, kepala seksi dan pemegang program. Penentuan prioritas masalah kesehatan dilakukan dengan menggunakan metode USG berdasarkan kriteria Urgency, Seriousness, Growth dan pencarian akar masalah menggunakan metode fishbone diagram. Peningkatan Kasus HIV/AIDS dengan skor USG 128 menjadi masalah kesehatan prioritas utama di Kabupaten Pacitan. Peningkatan selama 4 tahun terakhir dengan jumlah kasus tertinggi pada tahun 2019 yaitu sebanyak 39 kasus. Diagram fishbine menunjukan akar masalah HIV/AIDS yaitu kurangnya pengetahuan masyarakat terhadap HIV/AIDS, Kurangnya kesadaran penderita berisiko untuk melakukan pemeriksaan tes HIV, Hal ini membuat masyarakat kurang mengetahui informasi tentang HIV/AIDS sehingga menimbulkan stigma masyarakat yang buruk dan mengakibatkan masyarakat tertutup/tidak mau memeriksakan dirinya ke puskesmas ataupun rumah sakit. Peningkatan kasus HIV/AIDS adalah salah satu masalah di kabupaten Pacitan. Untuk menekan angka kejadian, petugas kesehatan perlu mengoptimalkan penyebaran informasi mengenai HIV/

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AIDS khususnya faktor risiko, penyebab, pencegahan, gejala yang timbul dan pengobatanya. Meningkatkan pemahaman petugas kesehatan dan kesadaran masyarakat dalam melakukan deteksi dini.

Kata kunci: *HIV/AIDS, Urgency, Seriousness, Growth*

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INTRODUCTION

In an effort to improve health status and the implementation of health development in Indonesia, there are various challenges, including the problem of inequality in public health status, access to health services, socio-economic level, and so on. In addition, new challenges arise as a result of socio-cultural, economic, and political changes as well as environmental changes. Health as a human right is explicitly mandated by the 1945 Constitution, which states that everyone has the right to live in physical and spiritual prosperity, to have a place to live, and to have a good and healthy living environment and have the right to health services. [1]

The implementation of health problem analysis is carried out to increase the effectiveness and efficiency of solving health problems through the selection of health problems that become priority problems in a region. By focusing on the selected health problems as a priority, it is hoped that the utilization of limited health resources can be carried out optimally in accordance with the leverage of the problem.

The results of the problem identification through a documentation study by comparing the program's achievements against the MSS targets, the Strategic Plan of the Ministry of Health and the RPMJD of Pacitan District and looking at trends for three consecutive years found nine main problems, namely HIV/AIDS, leptospirosis, hepatitis A, dengue fever, diarrhea, pneumonia, larva free rate, complete basic immunization and tuberculosis. Based on the results of data analysis and discussions with the head of the field and program holders, the priority of the health problem that was taken was HIV/AIDS.

HIV (Human Immunodeficiency Virus) is a virus that attacks the immune system. The infection causes the patient to experience a decrease in immunity so it is very easy to be infected with various other diseases. AIDS (Acquired Immune Deficiency Syndrome) is a collection of symptoms of reduced self-defense ability caused by the entry of the HIV virus. The HIV control program in Indonesia aims to: 1.) Reduce to eliminate new infections; 2) Reduce or eliminate AIDS-related deaths; 3) Reduce stigma and discriminationI. [2]

According to WHO, 2019 HIV can be transmitted through the exchange of various body fluids from an infected person, such as blood, breast milk, semen and vaginal fluids. HIV can also be passed from a mother to her child during pregnancy and childbirth. People cannot be infected through everyday contact such as kissing, hugging, shaking hands, or sharing personal objects, food, or water. [3]

HIV/AIDS is an infectious disease that occurs in the community for which there is no vaccine or effective drug for the prevention of HIV/AIDS until now.[4] According to World Health Organization (WHO) in 2018, there are 36.9 million people who have HIV/AIDS around the world.[5] Indonesia is one of the countries with the fastest addition of HIV/AIDS cases in Southeast Asia, with an estimated increase in the incidence of HIV infection by more than 36%. The HIV/AIDS epidemic in Indonesia is growing the fastest among Asian countries.[4] Indonesia occupies ranked third as a region with most people living with HIV/AIDS worldwide the world with a total of 5.2 million souls.[6] As of December 2019, the number of AIDS cases reported in East Java was 1,254 people, and 9,981 HIV cases. East Java Province is designated

as an area with concentrated HIV prevalence along with 5 (five) other provinces, namely DKI Jakarta, Papua, Bali, Riau and West Java.[7, 8] stated that HIV AIDS has become apandemic in Sub-Saharan Africa. HIV AIDS pandemic slowly causes a decrease in energy employment, reduce agricultural productivity, increasing poverty, and changing the structure population pyramid in Africa. Almost half of all HIV cases had no known risk factors (51.0%). Some of the highest risk factors are MSM at 20.4%, heterosexual 19.6% and IDU at 0.9%. While the highest AIDS cases were heterosexual at 73.4% and the lowest was transfusion at 0.3%. According to the type of work, the highest distribution of AIDS cases was among non-professional staff (employees) (26.4%), housewives (15.5%) and self-employed (12.6%). [2]

Based on the results of the problem analysis from the Pacitan Health Office, it was found that HIV/AIDS data in 2017 there were 36 cases then decreased although not so significantly in 2018 to 25 cases then increased again in 2019 to 40 cases.

The availability of health facilities in every sub-district in Pacitan Regency certainly makes it easier for the community to get access to better health services, if viewed from the distribution of sub-districts, there are already available puskesmas, an average of 2 units.[9]

The purpose of this study was to analyze the problem and determine the priority of health problems in the work area of the Pacitan District Health Office, East Java Province.

MATERIALS AND METHODS

Materials

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: Only rele-vant modifications should be described.

Methods

This research is a descriptive observational study conducted at the Pacitan District Health Office in January 2020. The type of data used is secondary data obtained from the 2016-2019 Pacitan District Health Profile and primary data obtained through interviews with related parties, namely the head of the field. , section head and program holder. The types of data collected are data on health status, population aspects, health behavior, environmental data and data on morbidity and mortality. Prioritization of health problems is carried out using the USG method based on the criteria of Urgency, Seriousness, Growth.

The steps taken in analyzing the health problems contained in the Blitar District Health Office are as follows:

1. Establish program achievement indicators using national/regional standards.
2. Comparing outputs on program achievements with indicators to look for gaps.
3. The method used for priority determination is the USG method. The USG method is one way to determine the priority order of problems using the scoring technique method.

These are as follows:

1. *Urgency*
   How urgently the problem must be discussed is related to the available time and how hard the time pressure is to solve the problem that caused the problem.
2. *Seriousness*
   How serious the problem needs to be discussed is related to the consequences arising from delays in solving the problem that caused the problem or the consequences that cause other problems if the problem causing the problem is not solved. It should be understood that under the same circumstances a problem that can give rise to another problem is more serious than a separate problem.

1. *Growth*
   How likely the problem is to develop is related to the possibility of the problem causing the problem to get worse if left alone.
2. There are many methods to find out the root cause of problems that arise in the workplace, one of which is fishbon. From the root of the problem found, then recommendations can be formulated for countermeasures that can be done to the problem.

RESULTS AND DISCUSSION

Overview of Health Problems in Pacitan District

Based on the results of the identification of health problems, examining the Pacitan Health Profile data for 2017-2019 and in-depth interviews and then comparing the program's achievements against the MSS targets, the Ministry of Health's Strategic Plan and the Pacitan District RPJMD, three main problems were found in Pacitan Regency as shown in Table 1.

Table 1. List of health problems in the Pacitan District in 2017-2019

No	Problem	2017	2018	2019	Tren	Target	Description
1	Number of HIV/AIDS Cases	36	23	39	increase	when there is a decrease case	HIV AIDS cases are still high
2	Number of Leptospirosis Cases	52	42	54	increase	when there is a decrease case	The number of leptospirosis cases is still high
3	Hepatitis A	0	0	1.314	increase	-	Hepatitis A outbreak occurs
4.	Dengue fever	72,1	48,3	121,9	Rising bad	-	The high number of dengue cases
5.	Diarrhea	54,8	56,4	3.26	Rising bad	RPJMD 100%	Haven't hit the target yet
6.	Penemonia	90,67%	92,64%	-	Rising bad	Renstra 60%	Hope < 60%
7.	Tubercolosis	199	328	-	Rising bad	-	Haven't hit the target yet

Prioritize problems

The method used is USG. The priority of the problem is determined by distributing the form based on the Urgency, Seriousness, and Growth criteria. The filling is carried out by the Head of Disease Prevention and Control, the Head of the Infectious Disease Section, the Head of the Surveillance and Immunization Section, the Head of the Non-Communicable Diseases Section, and all the staff of the Disease Prevention and Control Section. Priority selection of health problems with ultrasound criteria, the score used is prone to 1-5 according to the provisions of the researcher. Then the priority of the problem is scored, the greater the score indicates that the problem is becoming a priority problem. The following is a recap of the results of the problem assessment using the USG schoring technique in the work area of the Pacitan District Health Office which can be included in Table 2.

Table 2. Determination of Priority Problems based on Ultrasound Criteria

NO	Health Problems	Urgency	Seriuseness	Growth	Total	Prioritas
1	Number of HIV/AIDS Cases	45	45	38	128	I
2	Number of Leptospirosis Cases	43	45	38	126	II
3	Hepatitis A	47	44	33	124	III
4	Dengue fever	44	42	33	119	IV
5	Diarrhea	38	40	36	114	VI
6	Pnemonia	36	38	29	103	VII
7	Tubercolosis	41	37	38	116	V

Based on the results of the study documentation, Table 2 explains that the results of the priority problem determination activities carried out in Pacitan Regency, HIV/AIDS is the first priority problem. Where the number of cases is increasing and the death rate is still there, as well as the consideration of the head of the field through a discussion process, it is concluded that the main topic that is taken is the problem, namely the case of HIV/AIDS at the Pacitan District Health Office in 2019.

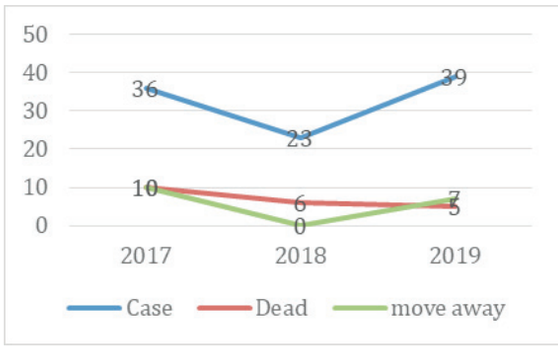

Figure 1. Number of HIV/AIDS Cases in 2017-2019

Figure 1 shows that in 2019 the number of HIV/AIDS cases as many as 39 cases increased dramatically from 2018 with 23 cases. During the last 3 years cases of HIV/AIDS deaths at the Pacitan District Health Office have decreased but are still high, in 2018 cases Deaths due to HIV/AIDS were 6 cases and 2019 were 5 deaths. Then the number who passed/moved from Pacitan Regency who were positive for HIV AIDS which was initially registered 0 cases in 2018 increased to 7 cases in 2019.

Table 3. Distribution of HIV/AIDS Cases by Gender

Gender	Year			
	2016	2017	2018	2019
Male	24	21	12	25
Famale	16	15	11	14

Table 3 shows that the distribution of the frequency of HIV/AIDS cases by gender in 2016 the number of men was more than women, namely 24 people, in 2017 the number of men was more than women, namely 21 people, in 2018 the number of men was higher than women namely 12 people, and in 2019 the number of men was still higher than women, namely 25 people. It can be concluded that the average number of HIV/AIDS sufferers from 2016-2017 was more men.

Table 4. Distribution of HIV/AIDS Cases by Age 2016 2019

	Number of cases			
Category	**2016**	**2017**	**2018**	**2019**
Age				
0-9 Month	0	0	0	0
1-10 Years	3	0	0	5
11-20 Years	0	0	0	0
21-30 Years	10	6	8	8
31-40 Years	14	9	5	13
>40 Years	11	21	10	14

Based on Table 4, the distribution of the frequency of HIV/AIDS cases by age in 2016 the highest was 31-40 years old and the lowest was 0-9 months and 11-20 years with 0 people, in 2017 the highest cases were age >40 year with 21 cases and the lowest was 0-9 months and 11-20 years with 0 people, in 2018 the highest number of cases was >40 years with 10 people and the lowest was 0-9 months and 11-20 years with 0 people, and in 2019 the highest cases were at the age of >40 years with 14 cases and the lowest cases were 0-9 months and 11-20 years with 0 people. It can be concluded that the most HIV/ AIDS sufferers are >40 years old.

Identifying the Root of the HIV-AIDS Problem

After determining the priority of the problem using the USG method and discussing with the Head of Disease Control and Prevention and the Section Head for the Infectious Diseases section, HIV/AIDS is determined to be a priority problem, then proceed with compiling an Ishikawa diagram (fishbone diagram) to determine the root of the HIV/AIDS problem. in Pacitan Regency. The Ishikawa diagram was prepared together with the Head of Division, Head of the Infectious Diseases Section and the HIV/AIDS Program Holder.

Determination of the root of the problem in the priority of existing problems is done using 5M theory but the problems that exist in the HIV/AIDS program in Pacitan district are seen based on the influencing factors, namely Man, Method, and Measurement. From the results of interviews that have been carried out with the head of the field, the head of the infectious disease section and the holder of the HIV/AIDS program, the problem that causes HIV cases that are still high is the lack of public knowledge about HIV/AIDS, the lack of awareness of patients at risk for testing HIV/AIDS tests. This makes people less aware of information about HIV/AIDS, causing a bad public stigma and causing people to be closed/not willing to go to the puskesmas or hospital to check themselves. This is in line with the article published by the Pacitan PemKAB which states that it is necessary to increase knowledge about HIV to all elements of society so that awareness arises to reduce the incidence of HIV.[10] Most of the cases found by the Health Office are residents who work outside the city, have sexual relations not with partners, blood transfusions are unclear and drug users with injection needles.[11] This is in line with the local regulations of Pacitan Regency, In the chain of HIV transmission there are vulnerable populations, high risk populations, and infected populations. Vulnerable population is a group of people who due to their social environment, health status, resilience and family welfare, will be more easily infected with HIV. The population includes people with high mobility, teenagers, street children, and recipients of blood transfusions. [12]

Formulation of Alternative Problem Solving

Based on the results of determining the root cause of the problem using the Fish Bone Diagram, it is necessary to reduce HIV/AIDS cases in Pacitan Regency: Community participation as the management of various health efforts for individuals, groups and communities by involving the community in a planned, integrated, and sustainable manner. The goal is that the community is able to take advantage of the various health services needed independently in order to achieve the highest level of public health. This community participation includes two elements: 1) The holding of coordination meetings by stakeholders and the community (for example, representatives of key populations), the availability of funds allocated to civil society in efforts to combat HIV and AIDS, as well as capacity building (such as training and technical assistance) which is strategically followed as part of its planning, implementation and evaluation process (prites and posttests, 2) Easy access to health services (both general health and HIV and AIDS services).

DISCUSSION

HIV or Human Immunodeficiency Virus is a type of virus that attacks/infects white blood cells which causes a decrease in human immunity. AIDS or Acquired Immune Deficiency Syndrome is a collection of symptoms of diseases that arise due to decreased immunity caused by infection with HIV. Due to decreased immunity, the person is very susceptible to various infectious diseases (oprtunistic infections) which are often fatal. People with HIV need treatment with antiretroviral (ARV) to reduce the amount of virus ARV to prevent opportunistic infections with various complications.[13]

Acquired Immune Deficiency Syndrome (AIDS) is a retroviral disease caused by the HIV virus characterized by a decrease in the body's immune system, especially attacking T lymphocytes and a decreased CD4 count to less than 200 cells per L of blood or 14% of all lymphocytes regardless of clinical status. Normal CD4 count is 800-1200 cells per L of blood.[14]

Factors that are thought to influence the number of HIV and AIDS cases in East Java are the ratio of PDP services, the ratio of STI services, the percentage of poor people, the percentage of people who use condoms and the ratio of KT services. [15] Epidemiologically, the incidence of Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) has increased the mortality and mortality rate of the population at a young age. In addition, the

condition of HIV/AIDS can also damage the social economy, such as families can lose their livelihood arrangements, costs increase, and are a threat to national development and a challenge in managing the Millennium Development Goals (MDGs) the rate of transmission of HIV and AIDS. (IAKMI, 2013. [16]

Table 3 shows that people with HIV/AIDS starting from 2016-2019 tend to be more male. Related to the work done by men, more mobility outside the area. According to (17) An important aspect in the migration of Indonesian workers from the perspective of the spread of HIV/AIDS is that it involves families such as wives/husbands, children and migrant workers individually for a long time in the destination area or usually with a group of their same-sex colleagues. This has created a situation where male migrant workers in the destination area are usually motivated to visit localization or commercial sex workers (PSK). It is not a coincidence that the main concentration area for CSWs in Indonesia is also a concentration area for male migrant workers.

The incidence of HIV/AIDS is more common in risky sexual behavior as many as 16 cases (57.1 %). There is a significant relationship between traditional stakeholders and the incidence of HIV/AIDS, as evidenced by the P value of 0.014 (P < 0.05). Odds ratio 4 and CI : 1, 284 – 12, 468 indicate that respondents who engage in risky sexual behavior are 4 times more likely to suffer from HIV/AIDS than respondents who do not engage in risky sexual behavior. [16]

One of the root causes of the increase in HIV/AIDS is a lack of knowledge. On internal factors, information exposure can be influenced by age, social background, income level and education level of the respondent. This is in line with research, who is the ideal person to provide information about HIV/AIDS, most of them still answer friends or relatives (67% each) and 62 percent state that health workers are the ideal source of information.[18] Thus, it becomes input for stakeholders to increase the role of officers in providing information about HIV/AIDS to the community. Almost the same thing was stated about the level of knowledge of HIV/AIDS with pre-marital sexual behavior of students.[19] The results showed that low knowledge about HIV/AIDS can increase the vulnerability of young people to be infected. However, this study also found that the better the knowledge about HIV/AIDS, the greater the attitude of not supporting (rejecting) premarital sexual behavior.

From the root of the problem, one of them is the stigma of the community towards PLWHA. Many factors influence the occurrence of stigma on PLWHA in society. Health education that aims to increase knowledge about HIV/AIDS in many studies has been proven to be one of the most influential factors in reducing stigma.[20] Argued that the stigma against PLWHA which is influenced by the attitudes of family, neighbors, and community leaders is the source of certain perceptions (stigma) towards PLWHA, which is the most influential factor. Different things were stated by that the stigma given by people to PLWHA is influenced by age and education factors with educational factors having a greater influence. [21]

The results of the relationship or bivariate analysis using kai squared shows that there are four variables that has a significant relationship with the stigma of PLWHA (p value < 0.05), namely the respondent's perception of PLWHA, neighbor's attitude factor towards PLWHA, factor family attitudes towards PLWHA, and the character's attitude factors community towards PLWHA(22)

Based on research conducted in 24 puskesmas in 8 districts/cities, it shows that puskesmas officers are still not ready for activities related to STI and HIV-AIDS prevention services, both in terms of knowledge, skills and facilities that support these services. So it is necessary to conduct training to health workers about HIV-AIDS.[23]

Prevention with an integrated approach is highly recommended to create knowledge, attitudes, and awareness to control the spread of HIV/AIDS among young people. In carrying out HIV/AIDS prevention and treatment actions are influenced by perceived costs, namely perceptions of negative costs/aspects that prevent individuals from taking health actions including conducting HIV/AIDS checks and counseling. The only

special attitude in internal medical personnel related to HIV/AIDS as an effort to prevent the chain of transmission is the aspect of self-protection of medical workers and aspects of sterilization of medical devices. [13]

What has been done by KAB Pacitan to prevent HIV-AIDS, Communication, Information and Education is a process of delivering information (messages, ideas, ideas) about HIV and AIDS prevention and control from one party to another using information delivery media such as voice media. , print media and electronic media. [12]

HIV/AIDS prevention and control efforts are carried out through direct counseling at village meetings or at other meetings as well as through media such as radio broadcasts. Another effort is to secure donor blood by screening donor blood samples.[24] Based on existing data, there were 3,169 teenagers who received counseling, consisting of 1,424 boys and 1,745 girls, spread throughout Pacitan Regency. [25]

CONCLUSIONS

Based on the identification of health problems at the Pacitan District Health Office, the main problem in 2019 was HIV/AIDS with 40 cases and 5 deaths, an increase from the previous year. Problems that are still a priority problem in 2019 at the Pacitan District Health Office are HIV/AIDS, Leptospirosis, dengue fever, Hepatitis, diarrhea, peneumonia and Tubercolosis. Based on the analysis of the causes of the problem with the help of the Fishbone diagram in Pacitan Regency, several causes of the problem were contain of there is still a lack of public knowledge about HIV/AIDS, lack of awareness of patients at risk for HIV testing.

Based on this health problems , the recommendation of this research are first, increase and expand cross-sectoral and cross-programme collaboration, both government, NGOs, institutions, religious leaders, community leaders and existing communities in order to be able to prevent and control HIV/AIDS by conducting socialization. Second, the recommendation for the Health Office is to form a companion for HIV/AIDS sufferers in every Puskesmas to control patients. Optimizing training to increase health cadres. and KDS to eliminate community stigma and discrimination so that PLWHA are expected to open up at least to their families so they can support their treatment. Third, improving management systems, information, human resources, and health promotion.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Research Article

Antimicrobial Resistance Profile of MDR & Non-MDR Meropenem-Resistant *Pseudomonas aeruginosa* Isolates of Patients in Intensive Care Unit of Tertiary Hospital

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ABSTRACT

Pseudomonas aeruginosa is one of the Gram-negative bacteria that frequently causes infection of patients in the Intensive Care Unit (ICU) which is easily resistant to antimicrobial drugs. Patients infected with carbapenem-resistant P. aeruginosa are predicted to have a poor prognosis. This study aims to know the resistance profile of meropenem-resistant P. aeruginosa of patients in the ICU. The results of this study can be used as a measure on the success of antimicrobial resistance control, infection control programs and become a reference for empirical therapy in the ICU. This study used descriptive research and was carried out at the Clinical Microbiology Laboratory of Sanglah Hospital Denpasar for three years, from 2018 to 2020. The results showed 38 of the 93 isolates of P. aeruginosa in the ICU were resistant to meropenem and were derived from sputum and urine. The percentage of meropenem-resistant P. aeruginosa isolates was higher in the multi-drug-resistant group and mostly came from sputum specimens. In 2018, Non-MDR meropenem-resistant P. aeruginosa isolates was that 100% sensitive to all other antibiotics used to treat P. aeruginosa infections, including; ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin, and piperacillin-tazobactam. In 2019 no meropenem-resistant P. aeruginosa isolates were found. In 2020, its sensitivity to antibiotics ceftazidime and piperacillin-tazobactam was 20.0%, ciprofloxacin 60.0% and to antibiotics gentamicin and amikacin 100%. MDR meropenem-resistant P. aeruginosa isolates in 2018 were still sensitive to ceftazidime (15.4%) and amikacin (69.2%) antibiotics, while in 2019 they were only sensitive to amikacin (37.5%). In 2020, P. aeruginosa isolates were sensitive to the antibiotics ceftazidime and cefepime (11.1%), piperacillin-tazobactam (22.2%), and amikacin (88.9%). Amikacin may be the choice of treatment for MDR meropenem-resistant P. aeruginosa.

***Keywords**: Resistance Profile; Pseudomonas aeruginosa; ICU; Meropenem; Resistance*

ABSTRAK

Pseudomonas aeruginosa merupakan salah satu bakteri Gram negatif penyebab infeksi pada pasien di Intensive care unit (ICU) yang mudah resisten. Pasien terinfeksi P. aeruginosa yang resistan karbapenem diindikasikan memiliki prognosis yang buruk. pengendalian infeksi dan menjadi acuan pemberian terapi empiris di ICU. Penelitian ini menggunakan metode penelitian deskriptif dan dilakukan di Instalasi Mikrobiologi Klinik Rumah Sakit Sanglah Denpasar selama tiga tahun, dari 2018 hingga 2020. Hasil penelitian menunjukan 38 dari 93 isolat P. aeruginosa di ICU resistan terhadap meropenem dan berasal dari spesimen sputum dan urine. Presentasi isolat P. aeruginosa yang resistan meropenem lebih tinggi pada kelompok multi-drug resistan dan sebagian besar berasal dari spesimen sputum. Pada tahun 2018, isolat P. aeruginosa Non-MDR yang resistan meropenem, 100% sensitif terhadap semua antibiotik lainnya yang digunakan untuk terapi infeksi P. aeruginosa, antara lain ; ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin, dan piperacillin-tazobaktam. Pada tahun 2019 tidak ditemukan isolat P. aeruginosa Non-MDR resistan meropenem. Pada tahun 2020, sensitifitasnya terhadap antibiotik ceftazidime dan piperacillin-tazobactam 20,0%, ciprofloxacin 60,0% dan terhadap antibiotik gentamicin serta amikacin 100%. Isolat P. aeruginosa MDR resistan meropenem pada tahun 2018 masih sensitif terhadap antibiotik ceftazidime (15,4%) dan amikacin (69,2%), sedangkan pada tahun 2019 hanya sensitif terhadap antibiotik amikacin (37,5%). Pada

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tahun 2020, isolat P. aeruginosa sensitif terhadap antibiotik ceftazidime dan cefepime (11,1%), piperacillin-tazobactam (22,2%), serta amikacin (88,9%). Amikacin dapat menjadi pilihan terapi P. aeruginosa MDR resistan meropenem.

Kata kunci: *Profil Resistansi; Pseudomonas aeruginosa; ICU; Meropenem; Resistansi*

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INTRODUCTION

Pseudomonas aeruginosa is one of the gram-negative bacteria that is often found as a contaminant in hospitals.[1,2] This bacterium can be an opportunistic pathogen causing nosocomial infections in the blood, lungs, and other body parts after surgery, especially in immunocompromised patients, patient received appropriate medical procedure, invasive, surgical wound or burns.[2,3] Nosocomial infections are estimated to occur annually in 1.75 million hospitalized patients worldwide and result in 175,000 deaths.[4] *P. aeruginosa* accounts for 10%-20% of Hospital Acquired Infections in Europe.[5] The National Healthcare Safety Network (NHSN) reported, *P. aeruginosa* as the third most common gram-negative bacteria causing nosocomial infections during 2011-2014.[6,7]

Research by Ribeiro et al. from January 2010 to December 2013 found that *P. aeruginosa* was the second most common bacterium in the ICU Sao Paulo Hospital Brazil (14.5%), of which 48.7% was multi-resistant drug organism.[8] At Sanglah Hospital itself in the second half of 2020, *P. aeruginosa* ranked third highest bacteria that cause infection in the Intensive Care Unit (ICU) and High Care Unit (HCU).[9] Patients admitted to the ICU have a five to ten times higher risk of developing *P. aeruginosa* infection compared to patients admitted to other inpatients.[10] High frequency infection in the ICU is associated with a decrease in the patient's immunity due to the disease and the use of invasive devices such as catheters, nasogastric tubes, and ventilators.[3,10] *P. aeruginosa* has quorum sensing ability which is associated with the occurrence of biofilms on invasive medical devices in patients in the ICU.[1,2] The spread of *P. aeruginosa* infection through person-to-person contact is also more prone to occur in the ICU due to several factors a patient is combined in one relatively small room.[10]

In the management of infection therapy, the selection of empiric antibiotics in the ICU is not easy.[4] According to *Performance Standard for Antimicrobial Susceptibility Testing on Clinical and Laboratory Standard Institute* (2021), *P. aeruginosa* sensitive to antibiotics, beta-lactam combinations such as piperacillin tazobactam, 3rd generation cephalosporins especially ceftazidime, 4th generation cephalosporins (cefepime), aminoglycosides (gentamicin, amikacin), monobactams (aztreonam), carbapenems (except ertapenem) and fluoroquinolones (ciprofloxacin).[11] The aztreonam group often becomes resistant.[1] Treatment of infectious diseases caused by *P. aeruginosa* becomes difficult because *P. aeruginosa* is easily resistant to various types of antibiotics. The prevalence of *P. aeruginosa* resistance to antibiotics is higher in ICU patient isolates compared to non ICU patients.[10,12]

The irresponsible use of an antibiotic widely, repeatedly, and over a long period of time can lead to the emergence of antibiotic resistance.[10] The increase in treatment costs in cases of infection due to resistant bacteria is caused by various factors, including; patients get longer treatment, longer hospital stays, more intensive attention from health professionals such as doctors and nurses, or the use of newer antibiotics. Newer antibiotics generally cost more than older antibiotics. The potential for increased costs in overcoming cases of infection by resistant bacteria needs attention because it can increase the financial burden that must be borne by the state in the era of implementing the National Health Insurance program (JKN). [14]

Carbapenem is a type of beta-lactam antibiotic which has a broad spectrum of antibacterial

activity.[14] Carbapenems such as meropenem and imipenem are potential antimicrobial agents that also used to treat Multi-Drug Resistant *Pseudomonas aeruginosa* (MDRPA) infections.[14,15] Increasing resistance to carbapenem antibiotics is one of the phenomena that must be watched out for at this time. Patients infected with carbapenem-resistant *P. aeruginosa* are indicated to have a poor prognosis.[16]

This study aims to know the resistance profile of meropenem-resistant *P. aeruginosa* in the ICU. The results of this study can be used as a measure on the implementation of antimicrobial resistance control, infection control programs and become a reference for empirical therapy in the ICU.

MATERIAL AND METHODS

This study used a descriptive research and was conducted at the Clinical Microbiology Laboratory of Sanglah Hospital Denpasar for three years, from 2018 to 2020. Sanglah Hospital Denpasar is a tertiary referral hospital and the main health care center for the eastern part of Indonesia with facilities of 710 beds. The sample was clinical isolates of meropenem-resistant *P. aeruginosa* from patients admitted to ICU. All type of specimens were included in this study. Identification and antimicrobial susceptibility testing were conducted using the VITEK 2 automated system with GN card for identification and AST GN 93 for antimicrobial susceptibility testing, according to the 2020 Clinical Laboratory Standard Institute (CLSI) standard.[11,17] Data of antibiotic susceptibility test were collected and resistance profile was analyzed. Meropenem-resistant *P. aeruginosa* is a *P. aeruginosa* isolate with a minimum inhibitory concentration of ≥ 8 g/mL based on a dosage regimen of 1 gram every 8 hours.[11] The antibiotics assessed in this study were the antibiotics of choice for *P. aeruginosa* that were available and included in the Sanglah Hospital formulary, including piperacillin tazobactam, ceftazidime, cefepime, gentamicin, amikacin, and ciprofloxacin.[18] Multidrug-resistant (MDR) is a condition in which bacteria are resistant to at least one type of antibiotic from 3 antibiotic groups.[13] MDR *P. aeruginosa* is a *P. aeruginosa* isolate that is resistant to at least one of three or more classes of antibiotics of choice for this bacterium, including: quinolones (ciprofloxacin), extended-spectrum cephalosporins (ceftazidime, cefepime), penicillin (piperacillin tazobactam), aminoglycosides (gentamicin or amikacin) and carbapenems (meropenem).[11,13] The exclusion criteria were incomplete data on meropenem-resistant *P. aeruginosa* isolates from the ICU including the results of antibiotic sensitivity tests as well as data from other treatment rooms including the COVID-19 special ICU.

RESULTS

This study observe the sensitivity pattern of meropenem-resistant *P. aeruginosa* in 3 consecutive years, from 2018 to 2020. In general, from 2018 to 2020, 93 *P. aeruginosa* bacteria were isolated in the ICU Sanglah Hospital Denpasar. The number of non-multidrug-resistant (Non-MDR) *P. aeruginosa* isolates in the ICU showed an increasing number but the MDR isolates showed a decreasing number (Figure 1).

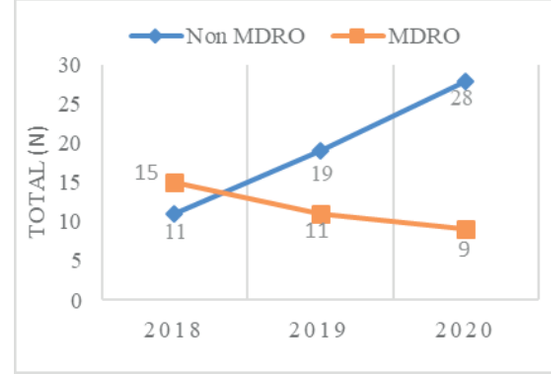

Figure 1. *Pseudomonas aeruginosa* in ICU during the period of 2018-2020

Most of the *P. aeruginosa* isolates came from sputum specimens, both Non-MDR and MDR, followed by urine and blood specimens. MDR *P. aeruginosa* isolates in 2018, 100% came from sputum specimens, while in 2019 and 2020, specimens came from sputum and urine specimens (Table I).

Table I. *Pseudomonas aeruginosa* in ICU during the period of 2018-2020 based on specimen

Specimen	2018 (%)		2019 (%)		2020 (%)	
	Non-MDR	MDR	Non-MDR	MDR	Non-MDR	MDR
Sputum	9(81.8)	15(100)	19(100)	9(81.8)	25(89.3)	8(88.9)
Blood	2(18.2)	0(0)	0(0)	0(0)	0(0)	0(0)
Urine	0(0)	0(0)	0(0)	2(18.2)	3(10.7)	1(11.1)
Total	11(100)	15(100)	19(100)	11(100)	28(100)	9(100)

Of the 93 isolates of *P. aeruginosa* in the ICU, 38 (40.9%) isolates had developed resistance to meropenem. The meropenem-resistant isolates were obtained from sputum and urine specimens. There were no meropenem-resistant isolates from blood. In 2018, 16 isolates were found from sputum, of which 3 of 9 (33.3%) were Non-MDR and 13 of 15 (86.7%) MDR. In 2019, 8 of 11 MDR isolates were resistant against meropenem, which was 66.7% in sputum and 100% in urine. In the Non-MDR group, no meropenem-resistant isolates were found. In 2020, the number of meropenem-resistant isolates were 14 isolates, 5 Non-MDR isolates came from sputum (20.0%) and 9 isolates from MDR, of which 8 isolates from sputum (100%) and 1 isolate from urine (100%). The percentage of meropenem-resistant *P. aeruginosa* isolates was higher in the MDR group (Figure 2).

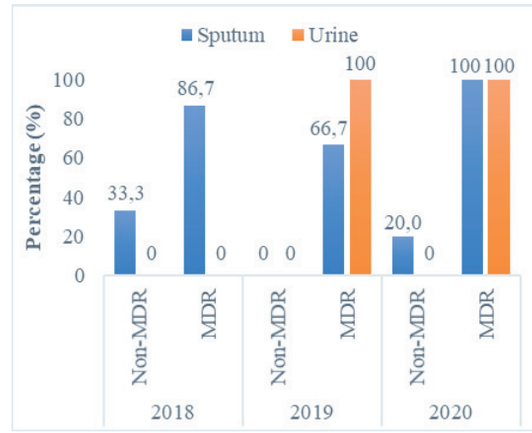

Figure 2. Meropenem-resistant *Pseudomonas aeruginosa* in ICU (N=38 isolates)

Based on table II, in 2018, Non-MDR meropenem-resistant *P. aeruginosa* isolates was 100% sensitive to all other antibiotics used to treat *P. aeruginosa* infections, including; ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin, and piperacillin-tazobactam. In 2019 no meropenem-resistant *P. aeruginosa* isolates were found. In 2020, the percentage of sensitivity of other antibiotics used to treat *P. aeruginosa* infection decreased dramatically compared to 2018, especially ceftazidime and piperacillin-tazobactam (20.0%), followed by ciprofloxacin (60.0%) while the sensitivity to gentamicin and amikacin was still 100%. MDR meropenem-resistant *P. aeruginosa* isolates in 2018 were still sensitive to ceftazidime (15.4%) and amikacin (69.2%). In 2019, MDR meropenem-resistant isolates of *P. aeruginosa* were only 37.5% sensitive to amikacin. The percentage was decreasing compared to 2018 and other antibiotics were already resistant. However, in 2020 the

Table II. Results of antibiotic susceptibility test of meropenem-resistant *P. aeruginosa* isolates in 2018-2020

No	Antibiotics	Non-MDR(%S)			MDR(%S)		
		2018(N=3)	2019(N=0)	2020(N=5)	2018(N=13)	2019(N=8)	2020(N=9)
1	Ceftazidime	3/3 (100)	0/0	1/5 (20.0)	2/13 (15.4)	0/8 (0.0)	1/9 (11.1)
2	Cefepime	3/3 (100)	0/0	1/5 (100)	0/13 (0.0)	0/8 (0.0)	1/9 (11.1)
3	Ciprofloxacin	3/3 (100)	0/0	3/5 (60.0)	0/13 (0.0)	0/8 (0.0)	0/9 (0.0)
4	Gentamicin	3/3 (100)	0/0	5/5 (100)	0/13 (0.0)	0/8 (0.0)	0/9 (0.0)
5	Amikacin	3/3 (100)	0/0	5/5 (100)	9/13 (69.2)	3/8 (37.5)	8/9 (88.9)
6	Piperacilin tazobactam	3/3 (100)	0/0	1/5 (20.0)	0/13 (0.0)	0/8 (0.0)	2/9 (22.2)

sensitivity of meropenem-resistant *P. aeruginosa* isolates to antibiotics improved, including to the ceftazidime and cefepime (11.1%), piperacillin-tazobactam (22.2%), and amikacin (88.9%).

The sensitivity pattern of MDR meropenem-resistant *P. aeruginosa* isolates (Table 3) showed that there was a pattern of MDR meropenem-resistant *P. aeruginosa* isolates that were also resistant to all antibiotics in the hospital. This pattern always exists every year with a fluctuating percentage and a significant decline in 2021. The pattern of MDR meropenem-resistant *P. aeruginosa* isolates sensitive to ceftazidime and amikacin antibiotics was only found in 2018. In 2021, the pattern of antibiotic sensitivity was more diverse. Most of them were resistant to various antibiotics, but each of these sensitivity patterns was still sensitive to amikacin antibiotics and one isolate of MDR *P. aeruginosa* was meropenem-resistant, besides being sensitive to amikacin, they were also sensitive to the ceftazidime, cefepime, piperacilin, tazobactam antibiotics. The Non-MDR meropenem-resistant *P. aeruginosa* isolate in 2018, the sensitivity pattern of 100% showed sensitivity to all anti-pseudomonal antibiotics available at Sanglah Hospital Denpasar. However, in 2020, the pattern of sensitivity to antibiotics varied because some antibiotics were already resistant.

DISCUSSION

Pseudomonas aeruginosa is one of the environmental bacteria that is often found in hospitals.[1,2] These bacteria can be opportunistic pathogens that cause nosocomial infections, including pneumonia, urinary tract infections, sepsis, osteomyelitis and skin infections including wounds and burns.[2] *P. aeruginosa* can grow in a variety of environmental conditions. Incidence of infection and resistance is common in the ICU. The bacteria found were often resistant to antibiotics.[10,19] Based on data from the antibiogram of Sanglah Hospital for the July-December 2020 period, *P. aeruginosa* was the third highest bacteria in the ICU and HCU.[9] The high rate of frequency of *P. aeruginosa* in the ICU is related to the decreased immunity itself, as well as the use of invasive devices such as catheters, nasogastric tubes and ventilators.[3,10]

Table III. Resistance profile of MDR and Non-MDR meropenem-resistant *P. aeruginosa* isolates

	Ceftazidime	Cefepime	Piperazilin tazobactam	Ciprofloxacin	Gentamicin	Amikacin	2018	2019	2020
No	**Meropenem-Resistant *Pseudomonas aeruginosa***						**MDR(%)**	**MDR(%)**	**MDR(%)**
1	S	R	R	R	R	S	2/13 (15.4)	0/8 (0.0)	0/9 (0.0)
2	R	R	R	R	R	S	5/13 (58.8)	3/8(37.5)	6/9 (66.7)
3	R	R	R	R	R	R	4/13 (30.8)	5/8 (62.5)	1/9 (11.1)
4	R	R	S	R	R	S	0/13 (0.0)	0/8 (0.0)	1/9 (11.1)
5	S	S	S	R	R	S	0/13 (0.0)	0/8 (0.0)	1/9 (11.1)
No	**Meropenem-Resistant *Pseudomonas aeruginosa***						**Non-MDR (%)**	**Non-MDR (%)**	**Non-MDR (%)**
1	S	S	S	S	S	S	3/3 (100)	0/0	1/5 (20.0)
2	R	R	R	R	S	S	0/3 (0.0)	0/0	2/5 (40.0)
3	R	R	R	S	S	S	0/3 (0.0)	0/0	2/5 (40.0)

According to the Performance Standard for Antimicrobial Susceptibility Testing at the 2020 edition of the Clinical and Laboratory Standard Institute, *P. aeruginosa* is sensitive to beta lactam combination antibiotics such as piperacillin tazobactam, 3rd generation cephalosporins especially ceftazidime, 4th generation cephalosporin (cefepime), aminoglycosides (gentamicin, amikacin), monobactam (aztreonam), carbapenems (except ertapenem) and fluoroquinolones.[11] The aztreonam group often becomes resistant.[1] Widespread, repeated, and long-term use of an antibacterial agent can lead to the emergence of antibacterial resistance.[15]

P. aeruginosa is intrinsically resistant to several antibiotics and has the ability to rapidly generate resistance to new antimicrobials. *P. aeruginosa* was the first bacterium to show an MDR phenotype.[8] Carbapenem antibiotics have become important in clinical management.[8,20] Carbapenem-resistant *P. aeruginosa* infections are common.[21] In February 2017, the World Health Organization made a priority list of pathogenic bacteria in the development of new antibiotics. Carbapenem-resistant *P. aeruginosa* is second ranked in the group of top priority (critical) bacteria because of its high resistance to most antibiotics including carbapenems and third-generation cephalosporins which are the best choices in the treatment of MDR bacteria.[22] In the United States, 10%–20 % of clinical isolates of *P. aeruginosa* identified in health facilities, resistant to at least one carbapenem group antibiotic.[23] *P. aeruginosa* became meropenem-resistant due to upregulation of the efflux pump.[24]

In this study, the percentage of meropenem-resistant *P. aeruginosa* isolates was higher in the multidrug-resistant group and most of them came from sputum specimens. Most of the sputum specimens collected in this study were from endotracheal tube secretions. *P. aeruginosa* has the ability of a bacterial cell-cell communication mechanism, known as quorum sensing (QS) which plays a role in gene expression and biofilm formation. The results of this study also support research in New York by Walter et al., that during July-October 2015 carbapenem-resistant *P. aeruginosa* was most commonly found in sputum specimens followed by urine.[18] Research by Asempa TE et al., in 2017-2018 also showed that 89% meropenem-resistant *P. aeruginosa* was found in respiratory specimens.[25]

The sensitivity of meropenem-resistant *P. aeruginosa* isolates varied to various antibiotics. Research carried out by Vitkauskienė A et al., in 2003 and 2008, isolates of *P. aeruginosa* that were resistant to carbapenems were more often resistant to ciprofloxacin and gentamicin than isolates sensitive to carbapenems. In 2008, isolates that were carbapenem-resistant were also more frequently resistant to ceftazidime, cefepime, aztreonam, piperacillin, and amikacin.[26] Results from the study by Asempa TE et al. in July-October 2017 showed that most of the carbapenem-resistant *P. aeruginosa* isolates had lower resistance to ceftazidime.[25] Research by Garcinuno et al. The data from 2009-2013 found that most of the meropenem-resistant *P. aeruginosa* isolates were also resistant to fluoroquinolones. Administration of amikacin therapy resulted in a more than threefold reduction in the risk of resistance.[27] Overuse of fluoroquinolones in the treatment of *P. aeruginosa* infections increased bacterial resistance to fluoroquinolones in recent years. Resistance to fluoroquinolones is mainly due to: (1) point mutations in the DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes, (2) the presence of transferable plasmid-mediated quinolone resistance (PMQR), and (3) mutations in genes that regulate efflux expression and decreased expression of outer membrane porins.[28] From the results of this study, in 2018 Non-MDR meropenem-resistant *P. aeruginosa* isolates were still sensitive to all antibiotics but in 2020 most of the sensitivity patterns showed sensitivity to gentamicin and amikacin. Although the MDR meropenem-resistant isolates of *P. aeruginosa* showed less sensitivity to various antibiotics, in 2020 the percentage of sensitivity to antibiotics except ceftazidime increased. Sanglah Hospital published Guidelines for the Use of Prophylactic and Therapeutic Antibiotics in 2019, ceftazidime is included in the Watch group of antibiotics. Ceftazidime not recommended as empiric

antibiotic therapy but should be based on the results of bacterial culture (definitive therapy) to *P. aeruginosa*, so its use restricted, in which allows the sensitivity *P. aeruginosa* to the ceftazidime increased in Sanglah hospital. This can be seen in the pattern of sensitivity, where although there is a pattern that is already resistant to all antibiotics every year, the percentage shows a fluctuating picture and significantly decreases in 2020. In addition, there were various other sensitivity patterns that showed MDR meropenem-resistant *P. aeruginosa* isolates were still sensitive to amikacin. The results of this study support the statement of Baseti et al. in 2018 which stated that all antipseudomonal antibiotics except amikacin were associated with the emergence of resistance in *P. aeruginosa*. Aminoglycosides modifying enzymes (AME) inactivate aminoglycosides by attaching acetyl, phosphate or adenyl groups to the amino and hydroxyl substituents on the antibiotic molecule. This modification significantly reduced the affinity of the aminoglycoside for the target of the 30S ribosomal subunit and blocks the activity of the aminoglycoside. However, compared to other aminoglycosides, amikacin is usually a poor substrate for this enzyme and is known to provide better antibiotic activity against *P. aeruginosa*.[28] The results of this study also support the study carried out by Khan F et al. in 2012-2013 regarding the Prevalence and Susceptibility Pattern of Multi Drug Resistant Clinical Isolates of *Pseudomonas aeruginosa* in Karachi and research by Anggraini D et al., regarding the Prevalence and Sensitivity Pattern of Multidrug Resistant Antimicrobial *Pseudomonas aeruginosa* in Arifin Achmad Pekanbaru Hospital in 2015 that amikacin is a therapeutic option for MDR *P. aeruginosa*.[29,30]

CONCLUSION

The resistance profile of meropenem-resistant *P. aeruginosa* in the ICU varies. Meropenem-resistant *P. aeruginosa* isolates that were Non-MDR for 3 years were still mostly sensitive to gentamicin and amikacin, while in multi-drug resistant isolates, amikacin was the choice of treatment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Original Article

Evaluation of Epidemiological Investigation 1-2-5 Implementation Program in Sukabumi

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ABSTRACT

Epidemiological investigations as part of the malaria surveillance system in Indonesia are carried out through the 1-2-5 method. Assessing the 1-2-5 strategy compliance level at the district level is the first step towards determining whether the surveillance and response strategy is working as planned or not. This study was conducted in order to determine whether PE 1-2-5 had been implemented in health centers (puskesmas) in malaria receptive areas according to the technical guidelines issued by the Indonesian Ministry of Health. Health centers were determined through purposive sampling technique. The sampling is determined by selecting health centers that have been doing malaria vector control service in 2018 and 2019. Ten Puskesmas in malaria receptive areas in Sukabumi District were selected. The informants in this study were the key players in the malaria program at the health centers: the head of the health centers, the manager of the malaria program, and the village malaria o cer (JMD) who were involved in the vector control process in 2018 and 2019 at the selected health centers. Data collection was conducted through in-depth interviews done by researcher with informants. The interview showed that the malaria program personnel in Sukabumi are doing the strategy as best as possible in order to achieve malaria elimination. 1-2-5 surveillance program in Sukabumi district has been implemented even though the implementation is not as ideal as the technical guidelines suggested by the Indonesian Ministry of Health, Sukabumi district still applied the strategy based on it by adjusting various aspects (resource situation and the availability of facilities) to the suitable condition in Sukabumi.

Keywords: *malaria, surveillance, epidemiological investigation, receptive, qualitative study.*

ABSTRAK:

Penyelidikan epidemiologi sebagai bagian dari sistem surveilans malaria di Indonesia dilakukan dengan metode 1-2-5. Menilai tingkat kepatuhan strategi 1-2-5 di tingkat kabupaten adalah langkah pertama untuk menentukan apakah strategi surveilans dan respon berjalan sesuai rencana atau tidak. Penelitian ini dilakukan untuk mengetahui apakah PE 1-2-5 telah dilaksanakan di Puskesmas di daerah rawan malaria sesuai dengan petunjuk teknis yang dikeluarkan oleh Kementerian Kesehatan RI. Penentuan Puskesmas dilakukan dengan teknik purposive sampling. Pengambilan sampel ditentukan dengan memilih Puskesmas yang telah melakukan pelayanan pengendalian vektor malaria pada tahun 2018 dan 2019. Terpilih sepuluh Puskesmas di daerah rawan malaria di Kabupaten Sukabumi. Informan dalam penelitian ini adalah pelaku utama program malaria di puskesmas: kepala puskesmas, pengelola program malaria, dan petugas desa malaria (JMD) yang terlibat dalam proses pengendalian vektor tahun 2018 dan 2019 di Puskesmas terpilih. Pengumpulan data dilakukan melalui wawancara mendalam yang dilakukan peneliti dengan informan. Wawancara menunjukkan bahwa petugas program malaria di Sukabumi melakukan strategi yang terbaik untuk mencapai eliminasi malaria. Program surveilans 1-2-5 di Kabupaten Sukabumi telah dilaksanakan meskipun pelaksanaannya tidak seideal juknis yang disarankan oleh Kementerian

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Kesehatan RI, Kabupaten Sukabumi tetap menerapkan strategi berdasarkan hal tersebut dengan menyesuaikan berbagai aspek (sumber daya). situasi dan ketersediaan fasilitas) dengan kondisi yang sesuai di Sukabumi.

Kata kunci: *malaria, surveilans, penyelidikan epidemiologi, reseptif, studi kualitatif.*

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INTRODUCTION

Malaria is a communicable disease that becoming a problem in many countries. In 2019, malaria has caused 409,000 deaths globally. [1] World health organization has reported that among all regions in the world, Southeast Asia is an area with a significant cost of malaria control. This significant impact has made malaria control becoming one of the government's main agenda in several countries, with malaria elimination as the final goal. [2]

The malaria Annual Parasite Incidence (API) in Indonesia is increased from 0.84 in 2018 to 0.93 per 1,000 population in 2019. At the provincial level, the provinces of Papua, West Papua and East Nusa Tenggara have very high malaria APIs compared to other provinces in Indonesia, 64.03, 7.38, and 2.37 per 1,000 population respectively. Most of the provinces (31 provinces) (91.2%) had malaria API < 1 per 1,000 population.[3] There are two ways of malaria infections, natural infection and unnatural transmission.[4] This makes the spread of malaria more rapidly, so it needs to be controlled.

Malaria control in Indonesia as stipulated in the Decree of the Health ministry of Indonesia aims to make Indonesia free from malaria transmission gradually until 2030.[5] The decline in malaria cases is an indicator of the government's success story in controlling malaria. This decline is expected to continue until the elimination of malaria is achieved. The indicator of malaria elimination in Indonesian district is shown by the absence of indigenous cases for three years consecutively in that district.[6]

Even though West Java Province is included into one of low transmission area when compared to the eastern Indonesia. This province still has not achieved its elimination state. In 2018, the West Java Provincial Health O ce reported a malaria morbidity rate which were 181 positive cases of non-indigenous malaria. Most of the cases came from Sukabumi, Garut, Tasikmalaya, and Pangandaran districts. In 2016-2019 the number of imported malaria cases reported in Sukabumi District were 111, 75, 76, 60, and 43 cases respectively. West Java Province is targeted to have eliminated malaria by 2024. In the year of 2020, West Java province is expected to be at the maintenance strategy stage. This makes every public health centre in a malaria receptive area demanded to be able to carry out surveillance and control of malaria vectors. [7]

Malaria elimination depends on a surveillance system that can detect, treat, and respond quickly and e ciently to individual cases in the population.[8] Epidemiological investigations (PE) as part of the malaria surveillance system in Indonesia are carried out through the 1-2-5 method depicted in table 1. The 1-2-5 method is one of the malaria control methods that contains things that need to be done after a positive case is found based on the processing time.[6]

One in the 1-2-5 method is defined as the first day a positive malaria case was reported based on microscopic examination, so after microscopic examination confirmed the case, the case must be reported to the district/city health o ce in 24 hours. Two is defined as the second day, it is the beginning of epidemiological investigation day which can be held from the second day to the fourth day. In this period, contact survey and risk factor observation such as case interview, vector survey, and the observation of people's behaviour should be conducted.[6]

Five is defined as the fifth day, the fifth day is the latest limit for the prevention to be carried out. Started with focus investigation. In this activity, risked population and vector presence are identified to clarify the source of infection. This activity followed by the making of case location map and the risk factor map present at that time. After that, focus countermeasures will be held. These countermeasures consisted of medication monitoring, searching for suspects who stays around the patient from one month before the confi rmed point, vector control (bed net distribution, IRS, larvicidation), health promotion, and the analysis of people's activity around breeding places (see in Table 1).[6]

Table 1. A series of epidemiological investigations and malaria's focus [6]

Day				
1	2	3	4	5
Alert Report (Notification)	Epidemiological investigation			Countermeasures
confirmed suspect	Case investigation			Focus investigation
↓	↓			↓
Positive	Contact survey and risk factor observation			Focus mapping
↓	↓			↓
Case report (1x24 hours)	Focus classification			Focus countermeasures

Similar control measures are also carried out in China by applying a 1-3-7 approach to monitoring malaria elimination strategies with activities such as case reporting, investigation and case management.[9,10] The "1-3-7" strategy in China has played an important role since the inception of the National Malaria Elimination Action Plan. This plan in detecting, treating, and responding to malaria cases has been running e ciently. This method is also proven to be able to eliminate the source of malaria infection faster. [11, 12]

Assessing the 1-2-5 strategy compliance level at the district level is the first step towards determining whether the surveillance and response strategy is working as planned or not. The absence of studies related to the implementation of PE 1-2-5 in malaria receptive areas in West Java Province is the basis for us to conduct research in one of the malaria receptive areas in West Java Province, Sukabumi district.

Even though West Java province were included into a low transmission for malaria when compared to the eastern Indonesia region. To conduct the 1-2-5 in low transmission also have its own merit as the low transmission is the first target of elimination. However, in community health centers, malaria might not be priority and complete for resources and manpower with other diseases. With existing health system and manpower, to properly adopt the epidemiological investigation 1-2-5 program based on technical guidelines issued by the Ministry of health might be questionable, it is important to conduct the study to evaluate the operation strength and weakness of the 1-2-5 method implemented in health centres in malaria receptive areas.

MATERIALS AND METHODS

Materials

This research is part of a descriptive study with a case study design titled "Determination of Vector Control Resources Indicators at the Community Health Centre (Puskesmas) Level in Malaria Receptive Areas in West Java Province".[7]

Research location is in Sukabumi district. Which in the southern region of West Java Province, is located at the coordinates of 6 ° 59'19 ″ South Latitude and 106 ° 33'03 ″ East Longitude. The administrative area of Sukabumi district is ± 419,970 ha or 4,145.7 km². Health centres were determined through purposive sampling technique. The sampling is determined by selecting health centres that have been doing malaria vector control service in 2018 and 2019.

The trend of malaria cases in Sukabumi in the last five years has relatively decreased from 111 cases in 2015 to 43 cases in 2019. In 2015, all puskesmas had a history of malaria cases both indigenous and imported. Puskesmas with more than 6 cases are Cisolok Puskesmas, Simpenan Puskesmas, Lengkong Puskesmas, Cimanggu Puskesmas, Tamanjaya Puskesmas, and Tegal Buled Puskesmas. In 2019, there were two health centers with more than three imported malaria cases, Puskesmas Cimanggu and Puskesmas Surade.

Based on the criteria, ten Puskesmas in malaria receptive areas in Sukabumi District were selected (Puskesmas Palabuhan Ratu, Puskesmas

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Cisolok, P uskesmas Cikakak, Puskesmas Lengkong, Puskesmas Simpenan, Puskesmas Jampangkulon, Puskesmas Cibitung, Puskesmas Tamanjaya, Puskesmas Surade, and Puskesmas Tegal Buleud) (Figure 1).

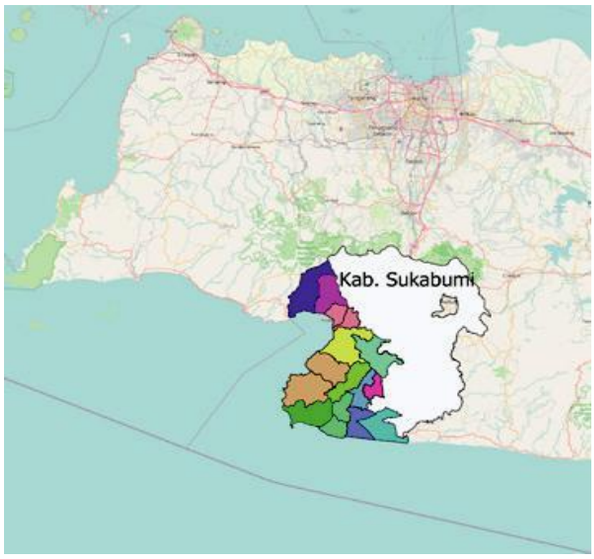

Figure 1. Research Locations in Sukabumi, West Java Province (The color di erence in Sukabumi districts shows the location of the Puskesmas (community health center) as the research location.

Informants

The informants in this study were the key players in the malaria program at the health centres, these key players have important roles in this research. The process of selecting informants was discussed in advance with the head of the health centres. After the informants were identified and willing to participate in this study, an agreement was made for data collection time. These key informants are the head of the health centres, the manager of the malaria program, and the village malaria o cer (JMD) who were involved in the vector control process in 2018 and 2019 at the selected health centres. The determination of the total informants' number is carried out by following the principles of adequacy and suitability that previously predicted by the researcher.[13] The number of informants from the health centres in Sukabumi was 28 informants.

Data collection and analysis

Data collection was conducted through in-depth interviews done by researcher with informants. Indepth interviews were conducted by a team of interviewers consisting of researchers from the Indonesian Ministry of Health, with educational background in public health and anthropology who have been trained and experienced in conducting in-depth interviews in qualitative research. Interviews were conducted one on one with each informant. The interview was conducted in a room determined by the informant, where the room used was a separate room from the workspace of other employees. This is intended so that topics that are confidential can be discussed by informants more freely. Interviews were conducted for approximately 1-2 hours. The completion of the interview was determined from the saturation of the answers from the informants after being asked the same rearranged questions.

The interview is limited to the topic of the implementation of PE 1-2-5 in each health center's work area. During the interview process, researchers were equipped with research instruments in the form of interview guides to guide in-depth interviews which developed from the elimination guide issued by the Ministry of Health [6]. The questions in the interview guide consisted of how the process of reporting malaria cases was carried out on 24 hours, how the process for case investigation was carried out on day 2, and how the case control process went and how long it took from reporting. Our in-depth interview also includes document observation in it.

The qualitative data analysis in this study is a thematic analysis. This analysis was carried out in three stages: the making of interview's transcript, data grouping and then the preparation of a matrix to see the relationship between each data group. Themes are produced based on case reporting, case investigation and case management. The transcripts of each response were examined and subjected to appropriate themes, then compared with other sources to identify repetition of words, relevant text, and phrases. Collective variation of

sources' opinions and views with related verbatim quotations is used to produce a narrative and outline of the findings.

Before the interviews were conducted, informants were provided with the consent form. All informants who agreed to sign the consent form were interviewed. Each informant was informed about the research based on the research explanation text. Informants were also informed about their option to stop the interview at any time without coercion. Informants who refused, gave up, decided to stop being interviewed and were not willing to be re-interviewed were excluded from the sample of this study. Each informant who was interviewed gave the researcher written consent to participate in this research. Data related to individual identities were removed from the data subset for ethical purpose. Ethical clearance for this research is obtained under the number LB.02.01/2/KE.475/2020 and issued by the Health Research Ethics Commission of the Indonesian Health Research and Development Agency.

RESULTS AND DISCUSSION

Result

Characteristics of informants

There were slightly more informants aged 40-60 years old than informants aged 20-40 years old, with most of them are men. Most of the informants' last education was university level. The number of informants by position in the health centres, as shown in Table 1, shows that not all selected health centres have JMD personnel (Table 2).

The Strength of 1-2-5 Implementation in Sukabumi

Based on the results of in-depth interviews with informants, malaria cases in ten puskesmas were reported to the Sukabumi District Health O ce on the day the cases were found or at least the following day. The report delivered by informal media, either by telephone or short message. If there is a case report, the program manager or JMD will confirm the patient and collect finger

Table 2. Characteristics of informants

Characteristics of informants	Total	Percent
Age group		
20-40 years old	13	46.43
40-60 years old	15	53.57
Gender		
Man	24	85,71
Woman	4	14,29
Last education level		
University	22	78,57
High School	6	21,43
Positions in Health centres		
Head of the health centres	10	35,71
Malaria programme manager	10	35,71
Village malaria o cer (JMD)	8	28,58

blood samples and coordinate with surveillance to report the case.

Although for the case of recording, the recording is applied informally by each informant individually (formal recording usually have a certain uniform format), each informant has their own way of recording the details of the case, but based on what this research observation, the case and case suspect finding are carried out within 24 hours based on key player's notes.

"yes, it is one day... once in 24 hours... if there is an information about a positive case, we are fast response, directly came to the places informed...we took their sample, we did the rapid test as well... JMD came with us... til to the puskesmas... if the result from the lab is there, then we confirmed it to the district health o ce... (informant 1)

"yes, we made the report in that day directly... well... actually it's depended... sometimes we did it the day after" (Informant 2)

"(in Sundanese) well... if there's a positive case... I think the report will be made the day after... through contact... by phone... the fi rst report made is... informal, I have to coordinate with surveillance o cer before anything else... (informant 3)

The informant stated that the investigation of the case (PE malaria) started on the second day. This case investigation is carried out by the program manager if microscopic examination results are out. Epidemiological investigations are carried out to probe information on whether the cases found are indigenous or imported cases, as well as to investigate the history of illness and to conduct a larva survey.

"Well... in the second day, we did PE to decide whether the case is indigenous or import..." (Informant 4)

"... The first thing we ask is the chronology... where did he/she came from, his/her origin transmit place, and then how long he/she has been stayed here... how long did he/she feel sick... previous sickness history... and then we will do the larvae survey in that location, mostly mr. H did it, I got the interview part... the result: if we found someone who's close to the patient and got a fever, usually the day after we will do MFS (Malaria Fever Survey) (Informant 2)

Apart from conducting PE, program managers and JMD also conduct contact surveys and risk factor investigations. A contact survey is carried out together with PE of malaria cases, mostly among family members. However, it does not rule out the surrounding community in a radius of 100 m from the case house. The contact survey is carried out by taking finger blood from family members or examining it using a rapid test.

"... and then we examined member of the household (who stay at the same house) who got closed contact with the patient. Even though If the house got two family in it, yes most of the house here is like that mam, one house usually have two to three family in it,,, eeehh, and we check all of them. If one of the members is positive, then we will do the widespread checking... for example, we check in 1 meter radius, ehhh I mean 100 meter wide upfront, 100 meters to the back" (informant 1)

Risk factors investigation is performed by observing the environment around the case whether there is a potential puddle of water as a breeding ground for Anopheles. If there is, then observation and Anopheles larvae collection are performed.

"Look at the local environment in behind and the front of patient house whether mosquito breeding places were present or not, if there isn't, maybe a rice field or a lagoon present in some distance"(informant 5)

"Then we also do the larvae collection... for JMD to determine whether the larvae were present or not..." (informant 4)

The fifth day activity in PE 1-2-5 is control cases formatted as treatment for patients. This activity is performed immediately after the case is confirmed (in the second or third day) and followed by monitoring starting from the fourth day. Monitoring of treatment is also performed by the Program Manager or JMD. In addition, counseling was also performed to the surrounding community.

"Yes, it was resolved immediately .. Followed up the case .. Treatment, the day after he was confi rmed positive. Yes, for example, we bring the medicine directly to his house if the test is positive, then we consult the doctor and just gave the medicine" (informant 6)

"(in Sundanese) well ... at least they were monitored we, we can follow up ... later ... after the treatment we do the blood test again..." (Informant 3)

"Positive ... the first thing we do is gave the medicine ... then they can go home ... and we told them that later in the 4th day we will come to their house ... in the 4th day, the 7th, and so on. (Informant 7)

"... later, if the result, for example, if there is someone who has closed contact with the patient has a fever, usually the next day we performed Mass Fever Survey" (informant 2)

"Eee In here, we gave the treatment directly. For example, if there are many suspects who are positive, maybe at least we gave them mosquito net. Promotion to promote the effort to the community, like counselling" (Informant 1)

The drawback of PE 1-2-5 implementation

The manager of malaria program in health centres encountered several drawbacks in implementing PE 1-2-5. One of those drawbacks is the absence of a standard reporting system for reporting suspect or case findings. This makes program manager report cases through informal channels such as short messages via messaging applications (WhatsApp) or by telephone.

" (in Sundanese) Well, at least if we found positive case, then the day after that... by that contact... we call them by phone before anything else... when about the report... we did it informally... before that I have to coordinated with surveillance programmer..." (informant 7)

"report by telephone or whatsapp right away that very day when we found the positive case..." (Informant 8)

Geographical drawbacks were also encountered in conducting PE 1-2-5 activities, especially when performing contact surveys.

".....generally, since this is a rural area, so the distance between houses were far. For example, when the procedure said in 200 meters perimeter, we only got few houses in that perimeter. Hehehe... So, in the end, we usually just came to the houses with mindset of getting at least twenty individuals or more". (Informant 2)

Other drawbacks include the availability of facilities and budget. Often the health centres did not perform a spot survey to identify risk factors due to the unavailability of the tools and costs needed to carry out a spot survey.

"... actually, we do want to do it, but we were limited by the unavailability of the tools... the tools to capture mosquitoes..." (Informant 4)

"... in order to do the spot survey, we were limited by its costs... there were no budget for that in BOK (Health Operational Assistance Budget) ... sometimes we did it by came along with o cer from district health o ce when they do those survey, sometimes we did it when the budget is in place... for spot survey..." (Informant 9)

In addition to contact surveys and risk factor investigations, usually population behavior surveys were conducted as well. However, most Health centres have never conducted a population behavior survey related to the risk of malaria transmission in their area.

DISCUSSION

Despite of its several constraints that need to be improved, the 1-2-5 surveillance programme in Sukabumi district has been implemented. Case and suspect case finding has been reported once within 24 hours even though it is done informally. This case reporting rate can minimize the increase in case transmission. Delays in malaria reporting in endemic areas can lead to

increased local transmission, as a result of delayed interventions in vector control and contact transmission surveys.[14] Therefore, through the 1-2-5 surveillance system, health centres are encouraged to report confirmed and suspected malaria cases within 24 hours to the district health o ce. Similar conclusion is reported from China, where confirmed and suspected malaria cases are reported within 24 hours using the web-based China Information System for Disease Control and Prevention (CISDCP).[15]

In contrast to malaria surveillance in China which has used a web-based information system, malaria cases reporting from the health center to the district health o ce is still performed informally, by telephone or messaging applications. However, quick reporting using telephone or short messages is considered more e ective than paper-based reporting. Reporting using a short message application such as WhatsApp allows the delivery of reports in real time, makes communication easier and allows the delivery of the same message (report) at the same time to several related parties (for example through the group feature). These advantages will allowed faster action/intervention process.[16, 17]

Meanwhile, the paper-based reporting system is considered formal but less than optimal because it requires limited media for distribution, communication, and human capacities. This paper-based reporting system is also not well integrated into every health service unit, such as in health centres and district health o ces.[18]

Although it is considered faster than paper-based reporting, reporting by telephone or short message still requires improvement, especially related to its reports form and information storage in the form of text and media (audio, visual, audiovisual). It is highly recommended to develop a web-based reporting system that comes with clear standard operating procedures. It might optimize the distribution of each report and it's archiving. Strengthening information systems and health monitoring is needed to avoid loss of information.[19]

It would be better if the malaria key player also consisted of individuals who specifically handled the prevention of malaria transmission. Unfortunately, this research still shows that this individual does not exist yet. All things related to malaria are still borne by the holders of the malaria program.

The case investigation is performed on the second day, consisting of two components, case confirmation and case classification. Based on the results of this study, it appears that all people with fever who come to health facilities or report to JMD, will be followed up by taking finger blood samples for microscopic examination or examination using a rapid test. In confirming this case, the ability of a laboratory officer to read blood slides had a significant effect. Trained laboratory personnel or personnel who work in high malaria endemic areas will have high probability to easily distinguish between positive and negative slides. However, in low endemic areas, this ability tends to be less used and forgotten. Therefore, the need for laboratory personnel certification in the confirmation of malaria cases is a necessity. Elimination of malaria will be impossible without access to adequate and well-trained human resources. [19]

Village malaria cadre plays an important role in malaria control, and this is the one of the strengths of malaria program in Sukabumi. Since malaria cadre is the representatives of the health center that are the closest to the community. Some of the JMD task include active and passive patient detection, epidemiological surveys, and vector control. [20] A study states that health cadres drawn from the community have an important role in the prevention, education and control of malaria cases in the community.[21] the first step to make JMD as one of the key players in the malaria program is increase JMDs knowledge regarding the tasks that they will carried out. Heads of Puskesmas and program managers play an important role in this. [22]

Case classification concluded from the results of interviews with the patient's disease history and mobility. Unfortunately, there is no confirmation from the medical records department and the puskesmas regarding unreported cases. A case is classified as imported only if the individual has traveled to a malaria endemic area in the previous month, else is classified as indigenous.

Classification of cases must be conducted carefully because it will affect the malaria elimination status in an area. Misclassification of cases can occur especially if interviews with suspected cases/ confirmed cases were not performed properly and in situations where population mobility between regions is high.[19] Regardless of whether cases are classified as indigenous or imported, case management must be performed in order to avoid the risk of local transmission, especially in receptive areas such as Sukabumi District.

Case control is performed within five days since the confirmed case or suspect is reported. The implementation of case management at the receptive community health center in Sukabumi district was in accordance with the technical guidelines, even before the fifth day, treatment had been given if the case had been microscopically confirmed. Management is not only limited to administering drugs in confirmed cases but also to follow-up treatment in the form of re-examination, and to perform counseling in the area where confirmed cases live. In fact, one health centre stated that if based on contact survey they found a person with symptoms of fever, it would be followed up by holding a mass fever survey (MFS). The implementation of this MFS is in accordance with malaria management guidelines. Additionally, then there are some health centres that distributes insecticide-treated bed nets.[23]

The drawbacks in implementing PE 1-2-5 raised by informants were mainly related to the facilities. Many informants mentioned the need to improve appropriate and adequate surveillance facilities. Unfortunately, the suitability and adequacy of the facilities are still not supported by qualified data. Despite of its importance in determining the condition of epidemiological readiness in an area, data collection regarding the capabilities and needs of health facilities in terms of malaria case management is still limited and minimal. A study in Uganda reported that knowledge and experience regarding the capabilities and needs for malaria case management were needed to increase the e ciency of the program.[24]

Other countries also experience drawbacks in implementing epidemiological investigation. Resources for investigating and responding to many cases especially in higher transmission and multiple pre-elimination arrangements are often too limited. Several countries in Asia Pacific region, members of the Asia Pacific Malaria Elimination Network, are considering adopting algorithms 1-3-7 for their own malaria elimination programs. In most cases implementing 1-3-7 approach will require an adjustment to the time frame. For example, in the Solomon Islands, reporting a case within 24 hours is often impossible due to lack of cell phone or internet coverage. Reporting of cases via VHF radio stationed at health posts can only take place within 48 hours, which means a "2-4-7" system may be more appropriate. In addition, 1-3-7 approach provide a simple set of targets for the applied program when assessing new elimination strategies, such as mass drug administration in response to outbreaks. A key component of the algorithm is the comprehensive and appositely recorded activities.[25]

The malaria control strategy in Indonesia still can not described as the successful strategy yet. A study conducted in Purworejo, Indonesia, reported the need for a change in strategy to achieve malaria elimination in the area. Indonesia has many challenges in implementing a malaria elimination program. Unrecapitulated data based on month, village, number of people with clinical malaria, age, sex, pregnancy status, plasmodium species, type of treatment and types of malaria transmission (indigenous or imported) make the control strategy less optimal.[26]

This study is inseparable from several limitations. This research is a cross-sectional study conducted in one period so that the concluded results cannot be generalized at all times. Moreover, this study also uses qualitative data that focuses on describing opinions and experiences in the last two years. Another limitation of this study is the small number of samples because this study is part of a qualitative study that uses the principle of adequacy as the basis for sampling.[13] The researcher determines whether the information extracted is su cient

enough to describe the malaria program in the study area or not. Therefore, the researchers determined informants who could explain correct and accountable information related to the malaria program in Sukabumi districts and some bias caused by opinion might be presented.

Despite of the existing limitations, to the best of authors' knowledge, this article is the first report that assessing the epidemiological investigation strategy implemented at the district level in Indonesia. This article can be used as a foundation for deciding whether the 1-2-5 strategy has been implemented according to the technical guidelines issued by the ministry of health.

CONCLUSIONS

The 1-2-5 surveillance programme in Sukabumi district has been implemented. Although the implementation is not as ideal as the technical guidelines suggested by the Indonesian Ministry of Health, Sukabumi district still applied the strategy based on it by adjusting various aspects (resource situation and the availability of facilities) to the suitable condition in Sukabumi. With this adjustment, hopefully policy maker could use this publication as a basis for the improvement of programme in order to prevent future malaria transmission.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Research Article

Description of Extraordinary Events of Dengue Hemorrhagic Fever In Belu Regency, East Nusa Tenggara Province 2020

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ABSTRACT

Belu Regency is located in the province of East Nusa Tenggara (NTT), Indonesia and is an endemic area for dengue fever. Nationally, until June 2020, there were 16,320 cases of dengue fever with a CFR of 0.009%, while in Belu Regency there were 820 cases recorded until June 2020 with a CFR of 0.97%. This study aims to describe the outbreak of DHF by person, place and time as well as the distribution of cases in Belu Regency. this research is descriptive observational with case series design. The source of research data is secondary data on dengue cases obtained from the 2016-2019 Dengue Hemorrhagic Fever (DHF) Report and the DHF outbreak report in January-June 2020, the Belu District Health O ce. DHF cases in Belu Regency until June 2020 were 820 cases with symptoms of fever 2-7 days by 100% and supported by laboratory platelet examinations of 73%. The highest IR rate until June 2020 is 367 per 100. 000 residents with a CFR of 0.97% spread over 12 sub-districts of Belu Regency. The highest IRs (>20 per 10,000 population) are Atambua city, South Atambua, East Tasifeto, West Atambua, Kakuluk Mesak and West Tasifeto sub-districts. The majority of DHF in the age group 5-14 years 521 cases (27.1%) with female sex as many as 495 cases (51.51%). DHF cases were found since the first epidemiological week at the beginning of the year with peak cases at the 13th week. Belu Regency Is a dengue endemic area with an IR of 367/100,000 population with a CFR of 0.97%. The highest cases were in the 5-14 year age group and spread across 12 sub-districts of Belu Regency.

Keywords: *KLB, DHF, Belu Regency, NTT Province*

ABSTRAK

Kabupaten Belu terletak di Provinsi NTT, Indonesia dan merupakan daerah endemis DBD. Secara Nasional kasus DBD hingga juni 2020 sebanyak 16.320 kasus dengan CFR 0,009% sedangkan di Kabupaten Belu tercatat hingga juni 2020 sebanyak 820 kasus dengan CFR 0,97%. Penelitian ini bertujuan mendiskripsikan KLB DBD menurut orang, tempat dan waktu serta sebaran kasus di Kabupaten Belu. Penelitian ini adalah deskriptif observasional dengan desain case series. Sumber data penelitian yaitu data sekunder kasus DBD yang diperoleh dari Laporan DBD tahun 2016-2019 dan Laporan KLB DBD bulan januari-juni 2020 Dinas Kesehatan Kabupaten Belu. Kasus DBD di Kabupaten Belu hingga juni 2020 sebanyak 820 kasus dengan gejala demam 2-7 hari sebesar 100% dan ditunjang pemeriksaan trobosit lab sebesar 73%. Angka IR tertinggi hingga juni 2020 sebesar 367 per 100.000 penduduk dengan CFR 0,97% yang tersebar di 12 Kecamatan wilayah Kabupaten Belu. IR tertinggi (>20 per 10.000 penduduk) adalah Kecamatan Kota Atambua, Atambua Selatan, Tasifeto Timur, Atambua Barat, Kakuluk Mesak dan Tasifeto Barat. Mayoritas DBD pada kelompok umur 5-14 tahun 521 kasus (27,1%) dengan jenis kelamin perempuan sebanyak 495 kasus (51,51%). Kasus DBD ditemukan sejak minggu epidemiologi pertama awal tahun dengan puncak kasus pada minggu ke-13. Kabupaten Belu Merupakan daerah endemis DBD dengan IR sebesar 367/100.000 penduduk dengan CFR 0,97%. Kasus tertinggi pada kelompok umur 5-14 tahun dan tersebar di 12 kecamatan wilayah Kabupaten Belu.

Kata kunci*: KLB, DBD, Kabupaten Belu, Provinsi NTT*

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INTRODUCTION

One of the infectious diseases that is still a public health problem in Indonesia is Dengue Hemorrhagic Fever (DHF). Dengue Hemorrhagic Fever (DHF) is a disease caused by the Dengue virus which is included in the genus Flavivirus, family Flaviviridae which has 4 types of sterotypes, namely Den-1, Den-2, Den-3, and Den-4 viruses. This virus can cause Dengue Fever (DD), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). DHF is transmitted to humans through the Aedes aegypti and Aedes albopictus mosquitoes.[1] Dengue Hemorrhagic Fever (DHF), if the fever is 2-7 days accompanied by bleeding manifestations, the platelet count is <100,000/mm^3, there are signs of plasma leakage (hematocrit increased by 20%), and or the results of the serological examination in a patient suspected of having DHF show a positive result or an elevation (positive) occurs. IgG alone or IgG and IgM in the dengue rapid test. Dengue hemorrhagic fever often causes extraordinary events so that it causes unrest in the community, because it is at risk of causing death and the spread of cases is very fast. In Indonesia, the first DHF case was reported in Surabaya in 1968 and then spread to all provinces and districts/ cities. In 2018, the number of DHF su erers was recorded at 65,602 patients, increasing to 138,127 patients in 2019. The number of patients who died also increased from 467 people to 919 people in the same period. Incidence Rate (IR) in 34 provinces in 2018 reached 24.75 per 100,000 population and increased in 2019 to 51.48 per 100,000 population. This figure is still above the national target of 49 per 100,000 population.[2]

The number of dengue cases in January to July 2020 reached 71,633 cases and in 2019 the number of cases was higher, amounting to 112,954 cases..With the number of deaths from January to July 2020 amounted to 459 people, while in 2019 there were 751 people.[3] Dengue Hemorrhagic Fever is still a health problem in both urban and semi-urban areas. In the Province of East Nusa Tenggara (NTT) dengue cases fluctuated from 2016 to 2019. In 2016, it was reported that there was an increase in the number of cases from 487 cases in 2014 to 1,213 cases. This number decreased in 2017 as many as 542 cases, increased in 2018 by 1,603 cases and continued to increase in 2019 by 4,059 cases. The incidence rate of dengue fever has also increased from 29.08/100,000 population to 74.39/100,000 population in 2019 so that it has not yet reached the 2019 NTT Provincial Health O ce strategic plan target, which is 8/100,000 population.[4] Belu Regency is one of the regencies in East Nusa Tenggara Province which is directly adjacent to Timor Leste, experiencing a significant increase in dengue cases. Where is from 2017 to 2019, it was reported that there was an increase in the number of cases from 6 cases to 101 cases in 2019. The incidence rate of dengue fever also increased from 1.4/100,000 population to 45.88/100,000 population.[5] Epidemiologically, the emergence of a disease is the result of the interaction between the host, agent and environment. Host refers to a human being who can become sick.[6] There are many factors that can cause the host to be susceptible to agent exposure, including age, sex, genetics, immunity, nutritional status and behavior.[7] The pattern of dengue transmission increases during the rainy season. Temperature and rainfall a ect mosquito breeding patterns.[8] Entering the rainy season in January 2020, dengue cases showed an increasing trend in almost all regencies/cities in NTT Province, one of which was Belu Regency. The data from the Belu District Health O ce noted that:Early January to June 2020, 820 cases have been reported and 8 of them have died. With an incidence rate of 367/100,000 population and a CFR of 0.97%. Many factors a ect cases of dengue fever which if without proper treatment will result in death. Various e orts to control the prevalence of dengue

cases, especially in areas with high or persistent transmission, are needed. Areas that have high transmission are cities/districts with high IR, so that requires careful and fast disease control.[9] One of the dengue control measures carried out in Indonesia and can be carried out by all ages and from all levels of education is the mosquito nest eradication activity. The government in Indonesia has launched the sustainable cultivation of mosquito nest eradication activity by the community with the core message of 3M plus and realizing the implementation of the 1 house one lartic monitor movement. The success of mosquito nest eradication activity activities can be measured by the larva free rate. If the larva free rate 95% is expected to prevent or reduce cases of dengue transmission. [10]

The purpose of this study is to provide an overview of the situation of dengue fever in Belu Regency and as input in e orts to prevent and control dengue cases in Belu Regency.

METHODS

This research is an observational descriptive study with a case series design. The data sources in this study used secondary data, namely the Health Profile of Belu Regency from 2016 to 2019, and DHF data from January to June 2020. This study describes the incidence of DHF cases with an epidemiological case approach according to person, place, and time. The variables studied in this study were Person (gender, age, clinical symptoms and incidence rate per age group), Place (case distribution and incidence rate based on Belu District), and time (seasonal pattern of DHF disease on a weekly, monthly and monthly basis) in Belu Regency. Age variables were grouped into 6, namely age groups 1 year, 1-4 years, 5-14 years, 15-44 years, and 45 years. The Incidence Rate (IR) variable is the result of the division between the number of new cases in a certain period and the total population in the area. The results of these calculations are then classified into 4 groups, namely, very high IR > 20%, high IR 16-20%, moderate IR 11-15%, low IR 6-14%, and very low IR < 5%. [11]

RESULTS

Dengue Hemorrhagic Fever Disease Pattern Based on Person (Clinical and Laboratory Symptoms)

The results of data analysis showed that in 2016-2020, the majority of cases of dengue fever showed clinical symptoms of fever 2-7 days by 100%, followed by symptoms of nausea/ vomiting, joint pain, headache and bleeding. Cases of dengue fever also from the results of laboratory examinations showed that the platelet count was <100,000 M3 by 100% in 2017 and 2018 while hematocrit >40% was the highest in 2020 by 91% and in 2016 by 60% (Figure 1).

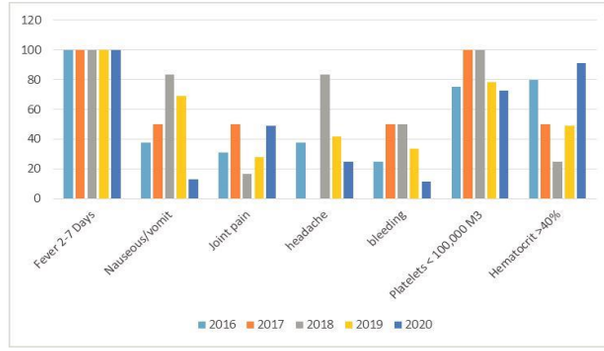

Figure 1. Distribution of DHF cases based on people according to clinical and laboratory symptoms in Belu Regency in 2016 – 2020

Dengue Hemorrhagic Fever Disease Pattern Based on Person (Gender and Age)

The results of data analysis showed that in 2016-2020, the majority of cases of dengue fever occurred in women. The pattern of distribution of dengue fever cases in 2016 to 2020, most su ered by the age group 5-14 years. The pattern of dengue fever incidence compared to gender which shows an increase every year is at the age of 15-44 years. The pattern of occurrence of dengue fever will decrease at the age of 45 years (Table 1).

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Table 1. Distribution of DHF Cases by Person (Gender and Age) in Belu Regency in 2016 – 2020

People Approach	Case (Year)					Amount	
	2016	2017	2018	2019	2020	n	%
Gender							
Man	13	0	2	45	406	466	48.49
Woman	19	2	4	56	414	495	51.51
Age (Years)							
1	2	0	1	2	20	25	1.30
1-4	3	1	0	21	130	155	8.06
5-14	22	1	3	31	464	521	27.11
15-44	5	0	2	39	172	218	11.34
>45	0	0	0	8	34	42	2.19
Total	**32**	**2**	**6**	**101**	**820**	**961**	**50**

Pattern of Dengue Hemorrhagic Fever by Place

The population density in Belu Regency from 2016 to 2020 has increased every year. Until 2020, the population in Belu Regency based on the Central Statistics Agency (BPS) is 223,176 people. Belu Regency is divided into 12 sub-districts and 81 villages (Table 2).

The incidence rate in Belu Regency shows an increasing trend every year. There was a decrease in the trend of cases in 2017 by 1/100,000 but increased in 2018 to 2020. In 2016 it was categorized as moderate IR, while in 2019 to 2020 it was categorized in a very high IR, namely 46 per 100,000 population and 367 per 100,000 population (>20 per 100,000 population) with a CFR of 0.97% (Table 3).

Table 2. Total Population of Belu Regency 2016 – 2020

subdistrict	Number of Villages/Sub-districts	Total population				
		2016	2017	2018	2019	2020
Raimanuk	9	14,428	14,355	14,262	14,166	14,040
Western Tasfeto	8	23,020	23.008	22,964	22,913	22,814
Kakuluk Mesak	6	3,307	3,188	3.068	2,952	2,834
Nanaet Dubesi	4	24,228	25,398	26,588	27,826	29.058
Atambua City	4	25,950	26,434	26,888	27,342	27,745
West Atambua	4	31.309	32,031	32,723	33,420	34,059
South Atambua	4	22,947	23,053	23,129	23,196	23,216
East Tasifeto	12	23,034	23,275	23,486	23,693	23,849
Take a look	6	20.551	21,953	23,418	24,974	26.575
Lasiolate	7	4.756	4,534	4,315	4,107	3,899
Lamaknen	9	6,530	6,376	6,217	6,061	5,895
South Lamaknen	8	10,247	9.991	9,725	9,466	9,192
Belu District	81	210,307	213.596	216,783	220,116	223,176

Source: Belu Regency BPS 2020

Table 3. Overview of Incidence Rate /IR and Case Fatality Rate /CFR DHF Belu Regency 2016 – 2020

Variable	2016	2017	2018	2019	2020
Total population	210,307	213.596	216,783	220,116	223,176
Number of Cases	32	2	6	101	820
Die	0	0	0	1	8
IR per 100,000	15	1	3	46	367
CFR	0	0	0	0.99	0.97

Source: Belu District Health O ce Profile 2016 – June 2020

The incidence rate value in several sub-districts shows an increasing trend, namely the West Tasifeto sub-district in 2018 IR 7 per 10,000 increased to 23 per 10,000 in 2019, Kakuluk Mesak District, IR in 2018 by 2 per 1,000 increased to 25 per 1,000. West Atambua Subdistrict in 2018 with IR 5 per 10,000 increased to 25 per 10,000 in 2019. East Tasifeto Subdistrict with IR 2 per 10,000 increased to 25 per 10,0000 in 2020. South Atambua Subdistrict in 2019 with IR 9 per 10,000 increased to 66 per 10,000 in 2020. Furthermore, Kota Atambua Subdistrict IR 8 per 10,000 in 2019 increased to 79 per 10,000 in 2020. So the classification of IR in 2020 where the IR is very low (<5) is Raimanuk District, Nanaet Dubesi District, Lasiolat District , Lamaknen District and South Lamaknen District. The low IR (6-14) is Raihat District. Meanwhile, those included in the very high IR (> 20) are West Tasifeto District, Kakuluk Mesak District, West Atambua District, East Tasifeto District, South Atambua District and Kota District (Table 4).

Table 4. Distribution of Incidence Rate /IR and Case Fatality Rate /CFR DHF per District Belu Regency 2016 – 2020

subdistrict	*Number of Cases (Year)*					*IR per 10,000 (Year)*				
	2016	**2017**	**2018**	**2019**	**2020**	**2016**	**2017**	**2018**	**2019**	**2020**
Raimanuk	0	1	0	4	7	0	1	0	3	5
Western Tasfeto	4	0	0	16	52	2	0	0	7	23
Kakuluk Mesak	3	0	0	7	72	9	0	0	2	25
Nanaet Dubesi	0	0	0	2	0	0	0	0	1	0
Atambua City	5	1	0	21	220	2	0	0	8	79
West Atambua	15	0	0	17	124	5	0	0	5	36
South Atambua	3	0	1	21	153	1	0	0	9	66
East Tasifeto	2	0	2	4	139	1	0	1	2	58
Take a look	0	0	1	5	18	0	0	0	2	7
Lasiolate	0	0	2	2	15	0	0	5	5	4
Lamaknen	0	0	0	2	15	0	0	0	3	3
South Lamaknen	0	0	0	0	5	0	0	0	0	1

Dengue Hemorrhagic Fever Disease Pattern Based on Time

Based on the weekly pattern of the outbreak in 2016 weeks 1-24 it can be seen that cases started in the first week and are still volatile, cases tend to increase at week 12 with peak cases at week 14. In 2017 and 2018 at week 11 and 5 with the tendency of cases to stop immediately. Whereas in 2019 and 2020 the initial cases began to occur in the first week where in 2018 the increase in cases tended to increase until week 5 with a peak of cases at week 9, and fluctuating at week 10-19 then increased again in week 21 and the trend of cases began to slow to week 26. While in 2020 the trend of cases increased at week 3 and continued to increase until a peak of cases occurred at week 13, however cases began to tend to decline at week 14 and continued to slope until week 26 (Figure 2).

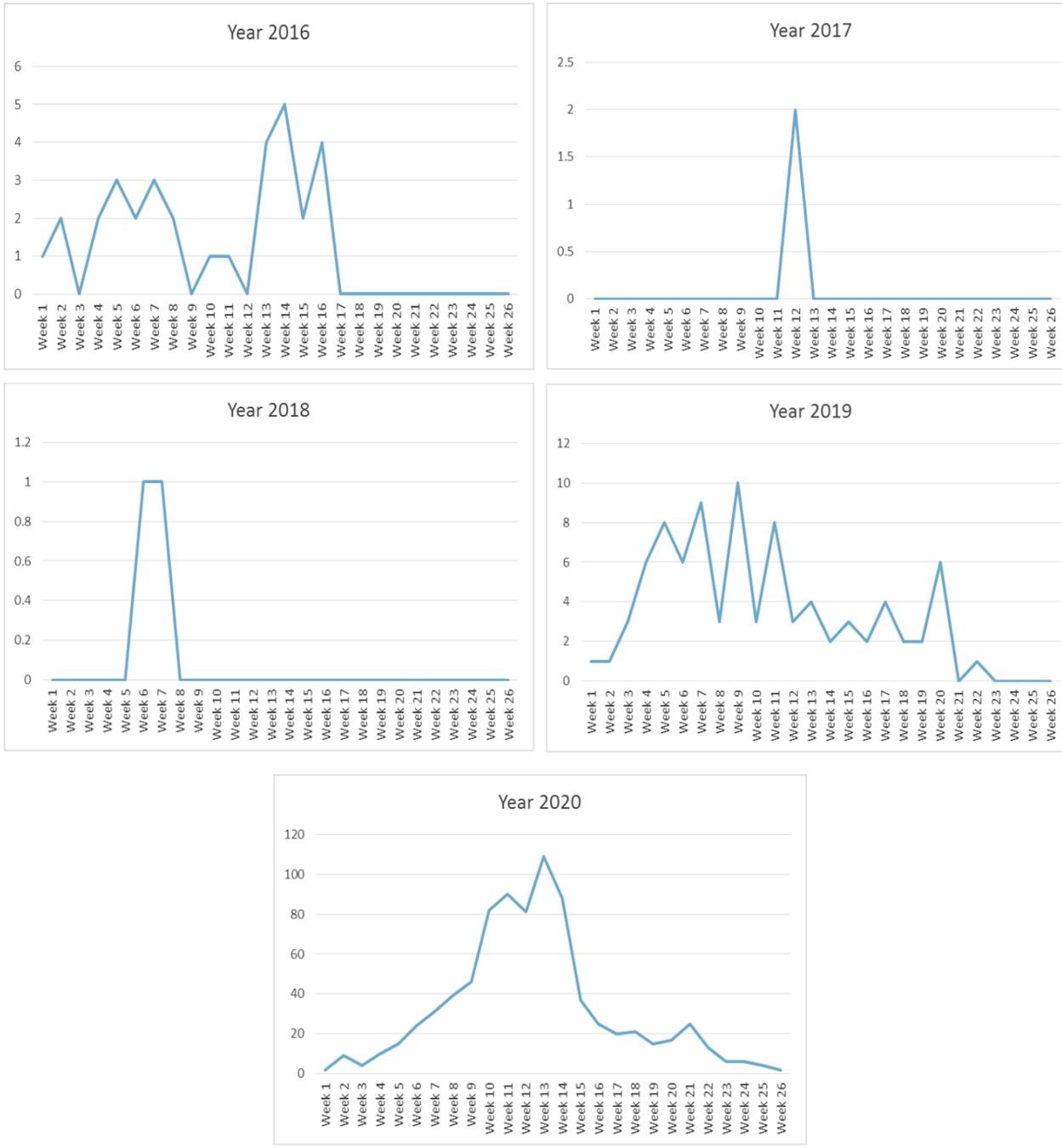

Figure 2. Weekly DHF outbreak week 1 - 26 in Belu Regency 2016 – 2020

DISCUSSION

Dengue Hemorrhagic Fever Disease Pattern Based on Gender, Age, Clinical Symptoms and Laboratory

A study conducted by a research team from Maranatha Christian University Bandung regarding the characteristics of DHF su erers in Kupang City found the fact that DHF mostly affects children under 15 years of age and more female su erers than males.[12] The same study on the characteristics of DHF patients at the Medan Haji General Hospital found that the most DHF patients were aged 11-20 years and there were more female patients than males.[13] Another study also stated that the gender group stated that the risk of developing DHF for men and women was almost the same, independent of gender.[14]

Another study with di erent results conducted in Banjarmasin found that DHF cases were more common in men (147 people) compared to women (98 people). Some of the di erences between the sexes of men and women, one of which is the mobility factor. Men basically spend more time outside the house, so the risk of being bitten by mosquitoes is even greater.[15] This study shows that the majority of cases of dengue fever occur in the 5-14 year age group. This study is in line with research conducted in Central Jakarta, Gambir sub-district and large rice field, which showed that the majority of dengue fever su erers occurred in the 15 year group.[14] This study is in line with research conducted in Thailand which showed that the majority of dengue fever su erers occurred in the 15 year group.[16] This study is in line with research conducted in Blitar City regarding the description of dengue cases which showed that the majority of dengue fever su erers occurred in the 15 year group.[17] Symptoms of dengue hemorrhagic fever are initiated by: 1) sudden high fever for 2-7 days (38oC-40oC); 2) hemorrhagic manifestations, with a positive tourniquet test, purpura, conjunctival bleeding, epitaxis, melena; 3) hepatomegaly; 4) shock, pulse pressure decreased to 20 mmHg or less, systolic pressure reached 80 mmHg or less; 5) thrombocytopenia, from day 3-7 found a decrease in platelets to 100,000/mm3; 6) hemoconcentration, increased hematocrit value; 7) other clinical symptoms that may accompany, anorexia, nausea, vomiting, weakness, abdominal pain, diarrhea, seizures and headaches; 8) and pain in muscles and joints.[18] This study showed that the majority of cases of dengue hemorrhagic fever with clinical symptoms of fever 2-7 days, nausea, vomiting, bleeding and decreased platelets <100,000/mm3. This study is in line with research conducted in Palu which showed that the majority of dengue fever su erers were fever 2-7 days, nausea and vomiting, and decreased platelet count <100,000/mm3.[19]

Pattern of Dengue Hemorrhagic Fever by Place

In general, the clustering of DHF events with a tendency to follow a high population density.[20] The distribution of dengue cases in Belu Regency tends to be concentrated in areas with densely populated settlements. It is recorded that the population has increased every year until 2020, which is 223,176 people. The incidence rate of Belu Regency in 2019 and 2020 is in a very high position >20%. This shows that the incidence of dengue cases in Belu Regency is very high. Belu Regency is an area with dense population mobility with a high population of people and is a direct border area with the State of Timor Leste. This study shows that the majority of dengue fever cases occur in high population and urban areas. This is in line with research conducted in Banyumas Regency where dengue cases are more widely spread in areas with a dense population such as the former Purwokerto City area with a population ranging from 2001 to 6885 people. In areas with a moderate population distribution between 1000-2000 people there are cases with a moderate distribution rate.[19] Another study conducted in China found that in the area of Guangzhou (one of the major cities in China), Conghua (its bu er city) and Zengcheng, more cases of DHF were found.[21] Urban and rural areas on the outskirts of the city are densely populated places so that the transmission of dengue virus through mosquito bites is more. Most of the residents in the new settlements have carriers of di erent types of virus carriers. An e ective intervention to overcome the spread of DHF is vector control. Even though the population is dense, if the vectors are few and not infective, the population will not be vulnerable.[22, 23]

Dengue Hemorrhagic Fever Disease Pattern Based on Time

In general, the pattern of cases increases during the rainy season and decreases in the dry season. This study showed that the increase in dengue cases occurred in the first week with peak cases in the thirteenth week with high rainfall. High rainfall causes puddles of water which are breeding places for mosquitoes that spread disease.[24] This is in line with research conducted in Surabaya which showed that rainfall was positively correlated with the incidence of dengue

hemorrhagic fever.[21] The same research was also carried out by Sumantri showing that areas/ locations with moderate rainfall, namely rainfall between 1000-1500 mm/year, while at high rainfall above 3000 mm/year cases were found to be few.[25] The pattern of increasing cases every month has a higher trend in December-May with reduced rainfall, especially in January. This condition is more due to the habit of residents to save water for household purposes, during times of water shortage.[20]

CONCLUSION

The incidence of DHF in Belu Regency all showed symptoms of fever for 2-7 days with the result laboratory examination of platelets <100,000 M3. The distribution of cases by gender mostly occurred in the female sex from 2016-2020. The pattern of DHF incidence based on age mostly occurs at the age of 5-14 years. The IR pattern in Belu Regency is relatively high because in 2016, 2019 and 2020 the IR rate was > 20 per 100,000 population with a CFR in 2020 of 0.97%. The highest IRs based on sub-districts in 2020 are Kota District, South Atambua District, East Tasifeto District, West Atambua District, West Tasifeto District and Kakuluk Mesak District. The pattern of DHF incidence based on time began to occur in the first week of the year and reached its peak at week 13 where during that time period the average rainfall was high. The pattern of DHF incidence is found in each year, the highest incidence occurs in January and February.

ACKNOWLEDGEMENT

This research uses secondary data obtained from the DHF report of the Belu District Health O ce and process eeed descriptively, so that the validity of the data in this research is very dependent on the validity of the data contained in the DHF report. In this research, the variables that became risk factors for DHF were not investigated, because they must be adjusted to the availability of data in the DHF report format. So it is hoped that further research can examine the risk factors for the incidence of DHF in Belu Regency.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Case Report

Convalescent Plasma Therapy: The Early Use in Moderate to Severe COVID-19 Patients in Hospitals with Limited Resources

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ABSTRACT

COVID-19 cases in Indonesia in the period of June-July 2021 showed a catastrophic spike. During this period, a recently discovered variant, the delta variant, appeared to be one of the sources of COVID-19 infection. Treatment modalities are limited due to reduced stock of drugs. A case of a 63-year-old man has been reported, with a history of having been vaccinated with two doses of Sinovac, experiencing moderate-to-severe symptoms of COVID-19 infection then given convalescent plasma therapy since his initial admission to the hospital. Three days after being given convalescent plasma therapy, the improvement was noticeable. Shortness of breath, cough, fever, and weakness were less complained. On the seventh day the patient fully recovered and got discharged. Convalescent plasma therapy was e ective in early stage and was able to improve outcomes. Indonesia needs su cient stocks of convalescent plasma as a therapy to overcome the limitations of medicines.

Keywords*: convalescent plasma therapy, COVID-19,infectious disease,health,*

ABSTRAK

Kasus COVID-19 di Indonesia pada periode Juni-Juli 2021 menunjukkan lonjakan katastropik. Selama periode ini, varian yang baru ditemukan, varian delta, tampaknya menjadi salah satu sumber infeksi COVID-19. Modalitas pengobatan terbatas karena berkurangnya stok obat. Dilaporkan kasus seorang laki-laki berusia 63 tahun, dengan riwayat pernah divaksinasi dengan dua dosis Sinovac, mengalami gejala infeksi COVID-19 sedang sampai berat kemudian diberikan terapi plasma konvalesen sejak pertama kali masuk rumah sakit. Tiga hari setelah diberikan terapi plasma konvalesen, terlihat perbaikan pada pasien. Sesak nafas, batuk, demam, dan lemas berkurang. Pada hari ketujuh pasien sembuh total dan diperbolehkan pulang. Terapi plasma konvalesen efektif pada tahap awal dan mampu meningkatkan hasil. Indonesia membutuhkan stok plasma konvalesen yang cukup sebagai terapi untuk mengatasi keterbatasan obat-obatan

Kata kunci: *terapi plasma konvalens, COVID-19, penyakit infeksi, kesehatan*

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INTRODUCTION

COVID-19 has turned out to be a global pandemic, with new variants currently emerging causing a spike in cases. In India, it was reported that since April 2021, there has been a massive escalation of new cases caused by delta variant, which has led to an increase of incidences, reaching more than 100,000 new cases with new deaths hitting almost 1000 cases per day. In Indonesia during June-July 2021, there also was a huge rise in COVID-19 cases and a delta variant was seemingly detected. New cases in Indonesia in mid-July 2021 have reached more than 40,000 cases per day with death cases reaching 1000 cases per day. Vaccination had been done to 5.5% Indonesian population per July 2021. Through the new wave of COVID-19 delta variant, Indonesia reported an average 919 deaths within first half of July 2021, statistically it is the same peak death rate in India at mid-May about 3.32 deaths per million people per day. Despite Indonesia new cases reached 56767 cases on July, it has low test positivity rate, 26%, that will mislead to many undiscovered cases.[1]

Many researches had been held across the globe to find the definite therapy for COVID-19. The therapeutic modalities in the COVID-19 treatment guidelines include symptomatic drugs, antivirals, steroids, and anti-IL6 blockers. On the other hand, there is still no guideline available in conduct the administration of convalescent plasma. IgG and IgM antibodies collected from patients who have been recovered from COVID-19, later it will be transfused to COVID-19 patient. The target therapy is increasing the chance to obtain neutralizing antibodies (Nab) against the virus.[2] Several studies have stated that convalescent plasma administration gives no significant result, otherwise, other studies and case reports announced that convalescent plasma is significant in moderate cases and should be given early.

In the following, we report a case of early administration of convalescent plasma therapy due to the limited availability drugs.

CASE

A man, age 63, initially complained of fever accompanied by sore throat. The patient then complained of nausea, vomiting, and diarrhea. The COVID-19 antigen test on the second day showed a positive result. On the third day, the patient had a cough and also complained of shortness of breath. Oxygen level on the third day of self-examination showed 97-98% room air, on the fourth day through the sixth day, the saturation slowly dropped from 93-94% to eventually become 88-91% along with worsening body temperature, cough and shortness of breath. The vital signs on admission: BP 120/80, pulse 110/minute, respiration rate 24 times/minute, body temperature 38.8˚C, O_2 Sat 92-93% free air, then given oxygen support with a 6 lpm simple mask and resulted O_2 Sat 97-98 %. The patient had no previous history of diabetes mellitus, heart disease, and hypertension.

The patient's medication history since the first day of symptoms was only symptomatic drugs plus Azithromycin 500 mg QD, Rebamipide 100 mg TID, Dexamethasone tablets 0.5 mg TID on the fourth day to the sixth day, and N-Acetylcysteine tablets 200 mg TID. The patient has been vaccinated with two doses of Sinovac vaccine. Laboratory examinations showed Hb 14.2 g/dl, lymphocyte 11%, neutrophil 82%, thrombocyte 171.000, leucocyte 6.260 u/L. AST 35u/L, ALT 37 u/L; blood sugar 113 mg/dl,urea 29.4 mg / dL, creatinine 1.16 mg / dL, C-Reactive protein (CRP) 20 mg/dl;IL-6 58.2 pg/ml; D-dimer < 0.2 mg/l. Blood gas analysis showed moderate to severe hypoxia; pH 7.45, pCO2 32, pO2 94, HCO3 22.2, BE - 1.0, SaO_2 99%, P/F ratio 235 (see Table 1). Chest x ray in ER showed ground glass opacity in both lung fields giving the impression of pneumonia. PCR test showed positive result.

Table 1. Initial Laboratory Result in Emergency Room and Day 6 in Hospital

Variable		Result day 1	Result day 6	Variable		Result day 1	Result day 6	Variable		Result day 1	Result day 6
Hb	g/dL	14,2	12,8	D-dimer	mg/L	<0,2	<0,2	Blood gas analysis			
WBC	10^3/uL	6,26	6,16	Blood glucose	mg/dL	113	110	pH		7,45	7,4
RBC	10^6/uL	4,42	4,13	AST	U/L	35	22	pCO2	mmHg	32	35
HCT	%	43	38	ALT	U/L	37	20	pO2	mmHg	94	88
PLT	10^3/ UL	171	235	Urea	mg/dL	29,4	20	HCO3	mmol/L	22,2	25
MCV	fL	89,4	88	Serum Creatinin	mg/dL	1,16	1,1	BE	mmol/L	-1	-1
MCH	pg	29,5	28	Electrolycte serum				SO2	%	98	98%
MCHC	g/dL	33	32	Na	mmol/l	132	131	P/F Ratio		235	440
Limfosit	%	12	7	K	mmol/l	3,49	3,8	FiO2		40%	20%
Monosit	%	5,8	5	Cl	mmol/l	102,9	103				
Eosinofil	%	0,1	0,1	IL6	pg/L	58,2					
Basofi	%	0,1	0,1	CRP	mg/l	20	10				
Neutrofil	%	82	88								

On the first day of care, The patient received Favirapir 1600 mg BID, intravenous Dexamethasone 6 mg QD, and symptomatic drugs. On the second day, antiviral drugs were no longer available, so the patient was given convalescent plasma therapy. Three days after convalescent plasma therapy, the breathing di culty and cough started to get resolved. The chest x-ray evaluastion was done that day. On the sixth day, the patient had no complaints, chest x-ray and laboratory examination was evaluated again (see Figure 1). The seventh day of treatment at the hospital, the patient was discharged with oxygen saturation 97-98% free air.

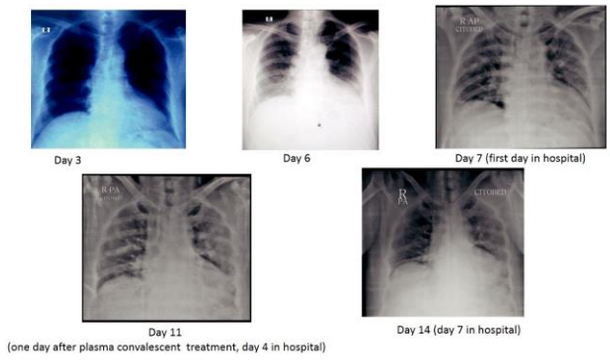

Figure 1. Chest X-Ray progression

DISCUSSION

COVID-19 has expanded globally. WHO recorded 190,770,507 confirmed cases of COVID-19 with 4,095,924 deaths in the world as of July 20, 2021. Meanwhile, Indonesia ever announced 2,877,476 confirmed cases of COVID-19 with 73,582 deaths and 2,261,658 patients who recovered from COVID-19[3]. Thus far, there is no yet definitive therapy for COVID-19. The management of COVID-19 currently focuses more on general supportive therapy and treatment of critical conditions.[4]

Numerous researches have been conducted to examine the therapeutic e ect of existing drugs on the severity of COVID-19, where previously these drugs were used to treat other diseases. Some drugs do not have a significant therapeutic e ect on the progression of COVID-19. The idea of passive immunization using convalescent plasma therapy emerged as a therapeutic option for COVID-19. Convalescent plasma is processed from the blood of donors who have been infected with a specific pathogen, such as SARS-CoV-2, whose plasma has formed humoral immunity and specific antibodies against that pathogen. This blood plasma also contains anti-inflammatory

cytokines, blood clotting factors, and other neutralizing antibodies (NABs) that are beneficial for regulating recipient immunomodulation. A study in China involving 10 patients with severe COVID-19 showed a decrease in viral load and clinically significant improvement in patients transfused with convalescent plasma concurrently with antiviral and other supportive therapy.[5] Another study conducted in Indonesia on 5 moderate COVID-19 patients and 5 severe COVID-19 patients manifested clinical improvement after three convalescent plasma transfusions at a dose of 3 ml/kg recipient's body weight at 2-days interval. Convalescent plasma therapy is more e ective when given early in disease progression. [6-10]

The main mechanism of the pathogenesis of COVID-19 is the occurrence of SARS-CoV-2 replication in the early phase and the occurrence of immune system dysregulation or inflammatory response to SARS-CoV-2, which results in tissue damage which occurs in the late phase. Based on this comprehension, the NIH recommends that COVID-19 treatment aims to destroy SARS-CoV-2 directly in the early stages, while administration of immunosuppressants or anti-inflammatory therapy will provide better e cacy if given in the later stages of COVID-19. The severity of COVID-19 will a ect the e ect of the therapy; inpatients without oxygen supplementation are not recommended to receive dexamethasone or other corticosteroids, in hospitalized patients with supplemental oxygen, remdesivir and/or dexamethasone may be considered [11] . Patients with a very rapid increase in oxygen demand may be given Baricitinib or Tocilizumab. Convalescent plasma therapy has not yet been included in the NIH recommendations.[12] The Food and Drug Administration (FDA) once issued a recommendation regarding the provision of convalescent plasma therapy in Emergency Use Authorization (EUA), which was then renewed again in February 2021. The FDA does not recommend giving convalescent plasma therapy with low antibody titers. This therapy is also not recommended in mechanically ventilated patients except in clinical trials. However, several observational studies have demonstrated a favorable therapeutic response to the use of high-titer convalescent plasma in patients with primary and secondary humoral immunodeficiency, including patients with haematological malignancies, agammaglobulinemia, and organ transplants.[13-16]

The current guidelines of COVID-19 treatments are formulated based on the recommendations of the WHO, CDC or medical organizations in each country. The University Hospital Birmingham Foundation Trust (UHBFT) in the UK developed the COVID-19 Quick Glance Guide in adults, which was updated in February 2021. UHBFT recommends general therapy such as empiric antibiotics for bacterial co-infection, thromboplophylaxis, fluid administration, antiviral remdesivir, single dose tocilizumab, and administration of dexamethasone. Dexamethasone can be given 6 mg orally or intravenously in patients who require oxygen supplementation or are on a ventilator, with the administration of gastroprotectant drugs. UHBFT also recommends supplemental oxygen and the proning position [17]. The Australian COVID-19 treatment guidelines do not recommend convalescent plasma therapy as standard COVID-19 therapy in pediatric patients, adolescents, adults, pregnant and lactating women, geriatrics and palliative patients, due to the finding that convalescent plasma therapy did not provide a more prominent therapeutic e ect than standard COVID-19 therapy.[18]

Guidelines for the management of COVID-19 in Indonesia have undergone various emendations along with the emergence of new studies during this pandemic. Five professional organizations consisting of the Indonesian Pulmonologist Association/*Perhimpunan Dokter Paru Indonesia* (PDPI), the Indonesian Cardiologist Association/ *Perhimpunan Dokter Spesialis Kardiovaskular Indonesia* (PERKI), the Indonesian Internist Association/P*erhimpunan Dokter Penyakit Dalam Indonesia* (PAPDI), the Indonesian Association of Anesthesiologists and Intensive Therapists/ *Perhimpunan Dokter Anestesi dan Terapi Intensif Indonesia* (PERDATIN), and the Indonesian Pediatrician Association/ *Ikatan Dokter Spesialis Anak Indonesia* (IDAI) issued a recently revised protocol for the management of COVID-19 on July

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14th, 2021. The therapy given is still adjusted to the severity of COVID-19. Recommendations are given in the form of supportive therapy, oxygen supplementation as needed, proning position, administration of pharmacological therapy in the form of: administration of vitamin C, vitamin B, vitamin D, antiviral favipiravir or remdesivir, dexamethasone or other corticosteroids in severe cases and requiring oxygen therapy, anti-interleukin 6 such as Tocilizumab or Sarilumab especially in severe or critically ill patients, as well as LMWH or UFH anticoagulants. Other therapies can also be given such as antibiotics for cases with bacterial coinfection, as well as convalescent plasma therapy. By far, convalescent plasma therapy has not been included in the main COVID-19 therapeutic guidelines because it is still in the clinical trial stage.[19]

A study was conducted in the United States involving 3082 COVID-19 patients. The patient was given a convalescent plasma transfusion with three di erent titers. The signal-to-cut-o ratio of anti-SARS-CoV-2 IgG antibody was categorized into: low titer (<4.62), medium titer (4.62-18.45) and high titer (>18.45). The study evaluated the mortality rate of patients after 30 days of convalescent plasma administration, discovered 115 deaths from 515 patients (22.3%) in the high titer group, 549 deaths from 2006 patients (27.4%) in the moderate titer group, and 166 deaths from 561 patients (29.6%) in the low titer group. In patients not on mechanical ventilation, administration of low-titer convalescent plasma had a higher risk of death than administration of high-titer convalescent plasma (relative risk 0.66; 95% CI 0.48 or 0.91). Convalescent plasma therapy had no e ect on the risk of death in patients on mechanical ventilation (relative risk 1.02; 95% CI 0.78 to 1.32). Patients who received convalescent plasma therapy within three days after being COVID-19-confirmed had a lower risk of mortality than patients who received transfusions at a later stage.[20] With some research data showing a fairly good response to high-titer convalescent plasma therapy, it can be considered as one of the recommendations for COVID-19 therapy.[21-25]

CONCLUSION

The administration of convalescent plasma at early stage of moderate-to-severe condition o ers ameliorate outcome. Therefore, in the midst of multiplying cases of COVID-19 in Indonesia as the consequence of new variants, it is considered principal to prepare convalescent plasma stocks as one of treatment options in addition to other medicines.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Research Article

Characteristics Environmental and *Anopheles* Larva Species In High And Low Clinical Malaria Cases In The Landak District of West Kalimantan Province

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ABSTRACT

Malaria remains a health problem in Indonesia. West Kalimantan is a malaria endemic area with high and low incidence. Landak District is one of the malaria endemic area. Malaria cases were found in the areas around illegal gold mining and oil palm plantations. The aims of this study were to describe the characteristics of the breeding sites and species of Anopheles larvae found in high malaria cases area, namely Amboyo Utara Village and low clinical malaria cases, area namely Mandor Village. This research is a descriptive research with cross sectional design. The samples were Anopheles larvae collected with Accidental sampling technique in the breeding sites. Environmental characterization of breeding sites were physical characteristic including water temperature and sun exposure, chemical characteristic including water pH and salinity, and biological characteristics including water biota. The results of this study ware environmental characteristics that have the potential to breed Anopheles mosquitoes in Amboyo Utara Village, including water temperature 26-30°C, shandy, water pH 5.0-7.6, salinity 0.2-1.0 ppt, biotas water hyacinth, grass and tadpole. The Mandor village, water temperature 29-30 ºC, shandy, pH of 6.9-8.0, salinity of 0.5 ppt, water biota grass. Anopheles species found in Amboyo Utara village were larvae of An. vagus (94.30%), An. tessellatus (3.42%), An. subpictus (1.62%), An. indefinitus (0.81%) and An. maculatus (0.81%). Characteristics of breeding sites in Mandor village were larvae of An. maculatus (11.11%), An. subpictus (3.70%), and An. vagus (85.18%). The conclusion of this study was that di erent species found at breeding sites with di erent environmental characteristics in both high and low malaria areas in Landak District, West Kalimantan Province.

***Keywords**: Environment, Larvae, Anopheles Species*

ABSTRAK

Malaria masih menjadi masalah kesehatan masyarakat malaria di Indonesia dan endemik Asia Tenggara. Kalimantan Barat termasuk daerah kasus malaria dengan insidensi rendah dan tinggi. Kabupaten Landak merupakan salah satu tempat kasus malaria yang terdapat di daerah lingkungan Pertambangan Emas Tanpa Izin (PETI), perkebunan kelapa sawit dan transmisi penularan malaria. Penelitian ini bertujuan untuk mennggambarkan karakteristik tempat perindukan dan spesies larva Anopheles yang ditemukan di daerah kasus malaria tinggi yaitu Desa Amboyo Utara dan daerah kasus malaria klinis rendah, yaitu Desa Mandor. Metode penelitian secara deskriptif dengan rancangan cross sectional. Sampel berupa larva Anopheles dengan teknik Accidental sampling. Hasil penelitian ini adalah Karakteristik lingkungan yang berpotensi sebagai tempat perindukan mengandung larva Anopheles di Desa Amboyo Utara yaitu suhu air 26-30 °C, teduh, pH air 5,0-7,6, salinitas 0,2-1,0 ppt, biota air eceng gondok, rumput dan kecebong. Desa Mandor yaitu suhu air 29-30°C dan teduh, pH 6,9-8,0, salinitas 0,5 ppt, biota

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air tanaman rumput. Spesies Anopheles yang ditemukan di desa Amboyo Utara yaitu larva An. vagus (94,30%), An. tessellatus (3,42%), An. subpictus (1,62%), An. indefinites (0,81%) dan An. maculatus (0,81%). Desa Mandor ditemukan yaitu larva An. maculatus (11,11%), An. subpictus (3,70%), dan An. vagus (85,18%). Kesimpulan penelitian ini adalah spesies yang berbeda ditemukan di tempat perindukan dengan karakteristik lingkungan yang berbeda pula baik di daerah kasus malaria tinggi dan rendah di Kabupaten Landak, Provinsi Kalimantan Barat.

Kata kunci: *Lingkungan, larvae, Spesies Anopheles*

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INTRODUCTION

Malaria remains a public health problem in Indonesia and endemic in Southeast Asia. Malaria is transmitted by the vector. The number of malaria patients in the world in 2015 is 216 million cases each year. A total of 655,000 people died from malaria.[1] Globally, in 2016 malaria in Indonesia caused the death of 445 thousand people. The decline in the last four years occurred in endemic districts in Kalimantan and Sulawesi.[2]

West Kalimantan Province is one of malaria endemis areas in Indonesia. The Equator line is crossed the province precisely in Pontianak City. The spread of the *Anopheles* mosquito from the equator where mosquitoes and mosquito breeding places adapt to the equatorial climate and environment (Depkes, 2009). Landak District West Kalimantan Province with three Sub-Districts which remain endemic to malaria were Ngabang sub-districts, Menjalin sub-districts and Air Besar Sub-District. Data on malaria cases in Landak District the last three years 2014, showed that 2,742 clinical malaria cases and 18 microscopic positive malaria cases. In 2015 there were 3,566 clinical malaria cases and 41 microscopic positive malaria cases. In 2016 there were 4,409 clinical malaria cases and 15 microscopic positive malaria cases. The Annual Parasites Incidence (API) of Landak District in 2016 was 0.04 per 1,000 population, and in 2017 was 0.03 per 1,000 population (Landak Sub-District Health O ce, 2017). The breeding places for *Anopheles* mosquitoes in this area includes puddles, ponds, and water gutters found in forests and oil palm plantations, rubber plantations, illegal gold mining, irrigation channels in rice fields, wells used for bathing, washing and other kinds of breeding places located near residential areas.

Amboyo Utara village that belongs to Ngabang Sub-District is area with high malaria cases in 2016 were 58 cases of clinical malaria and 15 cases of microscopic positive malaria microscopically. In 2017 the number of clinical malaria cases was 30 cases and 6 cases of microscopic positive malaria microscopically.[3] Low malaria cases in Mandor Village that belongs to Mandor Sub-district, in 2016 the number of clinical malaria cases ware 47 cases and in 2017 there were 24 cases and there were no microscopic positive malaria cases (zero).[4]

Amboyo Utara Village consists of rice field, vegetables and oil palm farming. Mandor Village consists of low land areas, most of which are farming, rubber plantation, and illegal gold mining. Some population activities have an impact on the health of the surrounding community, especially the onset of malaria disease. Forests logging that disrupt the natural habitat of *Anopheles* mosquitoes.[5]

Anopheles mosquitoes in Indonesia are found around 430 species, but 30-40 species of mosquitoes can potentially be a vector for malaria transmission in humans (Soedarto, 2011). *Anopheles* mosquito species that have been identified in West Kalimantan are *An. sundaicus*, *An. maculatus*, *An. letifer*, *An. balabacensis*.[6] Mosquito vector in the work area of Sekura Public Health Center, Sambas District Puskesmas Sekura, West Kalimantan is *An. campestris* with

breeding places in the lowlands, *An. nigerrimus* with a breeding place in the swamp and pond, *An. balabacensis* with a breeding place in a pond, a pool of water, a ditch, and *An. maculatus* with clear water and exposed to sunlight.[7]

Anopheles mosquitoes require a breeding place with certain physical, chemical and biological environmental characteristics. A research in Iran, reported that there is a relationship between habitat characteristics of *Anopheles* larvae and habitat environment with larval density. The program in vector control carried out in Iran is for malaria elimination.[8] Species of larvae have similar breeding sites in varying environmental characteristics with di erent locations of the study. [8]

Thes aims of this study were to describe characteristics of breeding sites in Mandor and Amboyo Utara villages associated with the species of *Anopheles* larvae found in both areas. This research was conducted in an e ort to understand the nature of *Anopheles* larvae in various breeding sites, in contributing to the efforts to control malaria vectors to prevent malaria transmission.

MATERIALS AND METHODS

Location

The area with high clinical malaria cases was Amboyo Utara village with located in Ngabang Sub-District, while area wih low clinical malaria cases was Mandor village. Which located in Mandor Sub-District, both villages were located in Landak District, West Kalimantan Province.

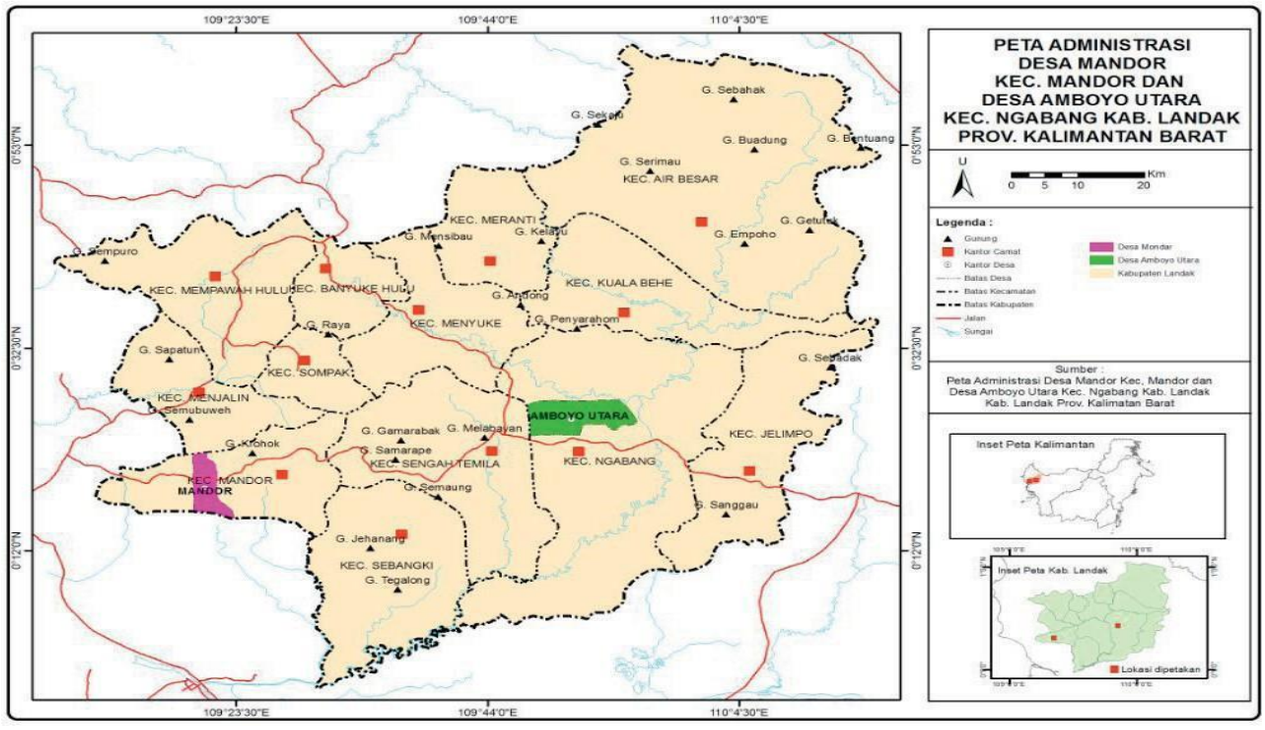

Figure 1. Amboyo Utara Villages is located in Ngabang Sub-District (green color) and Mandor Village is located in Mandor Sub-District (pink calor). Both are located in Landak District, West Kalimantan Province.

Research Subject

The subjects of this research were *Anopheles* larvae and their breeding sites found in Amboyo Utara village and Mandor village.

Sample Collection

The study was conducted in March 2018. The retrieval of *Anopheles* mosquito larvae was based on *Accidental sampling* techniques.[10] Arrest of

larvae is carried out at 07.00 - 17.00 WIB. *Anopheles* larvae in all breeding places locations in the two study villages. Larvae capture was carried out in accordance with WHO standards using a dipper device with a capacity of 320 ml. Identification of *Anopheles* larvae by using the keys of O'connor and Soepanto. [9]

Analysis data

Data were analyzed descriptively with a *cross sectional* approach.

RESULTS AND DISCUSSION

The survey results of breeding place types in Mandor village along with the characteristics of the environment in Amboyo Utara village in Table 1 and Mandor village in Table 2. The breeding place in North Amboyo village in Ngabang Subdistrict was found breeding sites containing *Anopheles* larvae, were water ditches, swamp, Excavation wells, rice fields, and drill wells. Mandor villages are found breeding places for illegal mining gold and Excavation wells.

The results of distribution of breeding characteristics in the environment that contained *Anopheles* larvae based on malaria cases were low and high in Table 1 and Table 2.

Table 1 showed that based on nine positive breeding sites of *Anopheles* larvae in North Amboyo Village, the characteristics of breeding sites in the physical environment with sun exposure of shady, temperatures of 25-30°C and shade in the village of North Amboyo.[23] temperature in the range 25-30°C the highest level of larval density is at 27°C in Karossa District, Mamuju Regency, Central West Sulawesi Province. Chemical environment at pH 5.0-7.6 and salinity 0.1-0.5 ppt in North Amboyo village. This study is in accordance with Mardiana and Perwitasari that 0 per mile of salt is found in the breeding place in the form of rice fields and ditches. Species found with a pH of 7-8 at potential breeding sites *An. vagus* positive in Sub-District Labuan, Pandeglang Sub-District, Banten Province.[11, 12, 15]

Biological environmental characteristics were found consisting of water hyacinth, tadpole and grass at the *Anopheles* larva breeding site in North Amboyo village. The presence of aquatic plants and leaves that fall in the breeding can be used by larvae to hide and obtain nutrients that contain nutrients that make larvae survive longer.[23] Tadpole (*Rana sp*) can be a predator but is a competitor to larvae. Some theories also say tadpole animals are aquatic animals that are herbivores that eat algae and other plants. However, there are also several types of tadpoles eating everything that can be eaten including larvae or animals.[18] The presence of animals and predators also influences the development and density of larvae, such as larva-eating fish, namely tin-head fish, tilapia and others.[12]

Table 1. Results of characteristics of breeding sites in the positive environment of *Anopheles* larvae in Amboyo Utara village, Ngabang District, Landak Regency.

No	Type of breeding placse	Amount of places breeding	Physical		Chemical		Water Biota	
			Sun Exposure	Temperature (°C)	pH	Salinity (ppt)	Animal	Plants
1.	Water ditch	1	Shady	26	7.3	0.5	-	Water hyacinth
2.	Excavation wells	3	Shady	25-29	5.0-7.5	0.1-0.4	-	-
3.	Swamp	3	Shady	26-30	5.7-6.6	0.1-1.0	-	Grass
4.	Rice fields	1	Shady	28	7.0	0.5	Tadpole	-
5.	Drill wells	1	Shady	26	7.6	1.0	-	-
	Amount Average	9	Shady	25-30	5.0-7.6	0.1-0.5	Tadpole	Hyacinth&grass

Table 2. Results of environmental characteristics with positive *Anopheles* larvae at breeding sites in Mandor village, Mandor Sub-district, Landak Regency.

No	Type of breeding placse	Amount of places breeding	Physical		Chemical		Water Biota	
			Sun Exposure	Temperature (°C)	pH	Salinity (ppt)	Animal	Plants
1.	Gold mining wells	1	Shady	30	6.9	0.5	-	grass
2.	Excavation wells	1	Shady	29	8.0	0.5	-	-
	Amount Average	2	Shady	29-30	6.9-8.0	0.5	-	grass

Table 2 showed that based on two positive breeding sites of *Anopheles* larvae in Mandor village illegal gold mining wells and excavation wells in a physical environment with water temperatures of 29-30°C and shady. The water temperature at the larval breeding site that is good for larval development is 28°C.[13] The optimum average temperature for mosquito development is 25-27°C.[14]

The chemical environment with a pH of 6.9-8.0 pH 7 is an ideal condition for the development of larvae and a mean salinity of 0.5 ppt because there is a low di erence in salinity in the breeding sites of gold mining wells and excavated wells due to the water environment contaminat by mercury. Biological environmentfound grass at the larvae breeding sites in Mandor village. Larvae take shelter in aquatic plants with many aquaticplants, moss grass thatis submerged in water.[17]

Table 3 showed that there were nine positive breeding sites containing *Anopheles* larvae as many as 123 in the Amboyo Utara village. Most species of *Anopheles* larvae were identified in the breeding sites in the dug fields *An. tessellatus* larvae 2.43%, *An. maculatus* larvae 0.81% and *An. indefinitus* larvae 0.81%, and *An. vagus* larvae

Table 3: Results of identification of *Anopheles* species at breeding sites in North Amboyo Village, Ngabang District, Landak Regency.

No	Types of breeding places	Amount of breeding places	Species	Amount of larvae (tail)	%
1.	water ditch	1	*An.indefinitus*	1	0.81
			An. vagus	2	1.62
2.	Excavation wells	3	*An. vagus*	19	15.45
			An. tessellatus	1	0.81
			An. maculatus	1	0.81
3.	Swamp	3	*An. vagus*	23	18.70
			An. tessellatus	1	0.81
			An. subpictus	2	1.62
4.	Rice fields	1	*An. tessellatus*	1	0.81
			An. vagus	64	52.03
5.	Driil wells	1	*An. vagus*	8	6.50
	Amount Average	9		123	100

52.03%, according to Mading (2014) in Selong Belanak Village, Central Lombok Regency. *Anopheles* larvae were found, including *An. vagus*, *An. subpictus*, *An. sundaicus*, *An. maculatus*, *An. aconitus*, and *An. anullaris*, in this research on the bioecological aspects. This contains *Plasmodium vivax* sporozoites which are thought to be malaria vectors to be able to ensure the need for surgery for mosquito saliva glands of *Anopheles* species.[17]

The results of the recapitulation of the species in the village of Amboyo Utara was *An. maculatus* larvae 0.81%, the number of these species that were found little larvae was

caused by the weather of the rainy season at the time of research in the village of North Amboyo. Density An. maculatus is high in the dry season, while in the rainy season the vector is somewhat reduced because the breeding places are a ected by rain so that the larvae are brought in by water when flooded. [17]
An. indefinit 0.81% was found in the ditch, according to stated for *An. indefi nitus* has habitat and lives breed in irrigation channels and rice fields that are close to the forest. There for this type of species is the least number of larvae found in breeding sites.[18]

Larva *An. tessellatus* larvae 2.43% was found in the Amboyo Utara of village. *An. tessellatus* is a malaria vector in Sri Lanka and has the potential as a vector in Kalimantan (Munif and Imron, 2010) and in Donggala, Central Sulawesi. An. tessellatus Larvae can be found in habitats protected from sunlight, fresh water that flows slowly, although relatively high salinity has been reported.[19-20] [8] An. subpictus larvae was found 1.62% of breeding sites in North Amboyo village. In Anopheles mosquitoes, some of the ecology on the beach which tends to havebrackish water is also a breeding ground for An. subpictus.[15]

An. vagus larvae 94.30% are found most in each breeding place in the village of Amboyo Utara.An. vagus is found to rest more in livestockcages compared to people's homes. In Vietnam, is found An. vagus with brackish water habitat and P. falciparum cannot develop in mosquitoes An. vagus.[16] Diferent research in Thailand, at An. vagus found P. falciparum and P. vivax able to live in freshwater habitats through salivary gland surgery and ELISA. This is a diference in strains or species due to ecological diferences in geographical distribution.

The spread of larvae in the breeding site is not evenly distributed on the surface of the water, but collected in closed places such as moss, floating water plants, garbage and grass - grass on the edge of a river or ditch. Fresh water is used as a breeding ground, is open and gets direct sunlight.[21] Larva species in Mandor Village and Amboyo Utara Villages found five types of larvae species *Anopheles* larvae that consisted of *An. vagus*, *An. subpictus*, *An. maculatus*, *An. tessellatus, and An. indefinitus* which is confirmed as an *Anopheles* species which acts as a transmission of malaria vectors in Indonesia.[22]

The results of the distribution of *Anopheles* larvae species in the breeding place with the number of larvae based on low clinical malaria cases in the area of Mandor Village, Mandor Subdistrict, Landak District, West Kalimantan Province in Tables 4.

Table 4 showed that identification of Ngabang Sub-District, Landak Regency, West Kalimantan Province in Tables 4.

The results of the distribution of Anopheles larvae species in North Amboyo village, *Anopheles* species larvae showed that there were 27 tail positive breeding sites containing 27 Anopheles larvae in Mandor village. The larvae found are not many at the breeding site because the breeding sites contain mercury and this type of groundwater storage is partly acidic and yellow soil which a ects the proliferation of Anopheles larvae. Residents of the settlement dig the ground to search for gold stones illegally so that gold mining well is a risk factor for malaria cases. The occurrence of malaria is an outdoor activity at

Table 4. Results of identification of *Anopheles* species at breeding sites in Mandor Village, Mandor Sub-District, Landak Regency

No.	Type of breeding place	Amount of breeding places	Species	Amount of larvae (tail)	%
1.	Gold mining wells	1	*An. maculatus*	3	11.11
			An. vagus	9	33.33
2.	Excavation wells	1	*An. subpictus*	1	3.70
			An. vagus	14	51.85
	Amount Average	2		27	100

night, this is the habit of some exophagic mosquitoes at night, gold workers sleeping at gold mine sites and rubber farm workers who cut rubber. Mosquitoes endophagic like to suck human blood inside the house, including bite of active *An. maculatus* between 21.00-03.00 pm.[27-28]

The results of the identification of *Anopheles* larvae species found the breeding sites in the gold mining wells were found by larvae *An. maculatus* 11.11% and *An. vagus* 33.33%, while larvae of digging wells were found larvae *An. subpictus* 3.70% and *An. vagus* 51.85%.[28] in a study in Aceh Besar District stated that *Anopheles* mosquito species found in larval breeding sites are *An. sundaicus*, *An. vagus*, *An. barbirostris*, *An. kochi*, *An. maculatus*, and *An. tessellatus* which is thought to have potential as a malaria vector.

There were two breeding sites that contained *Anopheles* larvae in the Mandor village, namely *An. maculatus* 11.11%. Mosquito *An. maculatus* can develop well in open waters both flowing and not flowing and in the form of stones or soil.[23] Larva *An. subpictus* 3.70%, this species has been recognized as an important malaria vector in Sri Lanka, so bionomics *An. subpictus* in every type of breeding place becomes transmission in India.[24] Larva *An. vagus* 85.18% at the breeding site in the Mandor village. Larva *An. vagus* which is often found in calm or slow-flowing water and brackish water.[25-26] Study states that breeding sites have potential *An. vagus* is positive for sporozoites as a companion vector to transmit malaria to humans in Labuan Subdistrict, Pandegelang Regency, Banten Province.

CONCLUSION

The conclusion of this study is environmental characteristics that have the potential to breed *Anopheles* mosquitoes in Amboyo Utara Village, including water temperature 26-30°C, shandy, water pH 5.0-7.6, salinity 0.2-1.0 ppt, biotas water hyacinth, grass and tadpole. The Mandor village, water temperature 29-30 ºC, shandy, pH of 6.9-8.0, salinity of 0.5 ppt, water biota grass Anopheles species found in Amboyo Utara village were larvae of An. vagus (94.30%), An. *tessellatus* (3.42%), *An. subpictus* (1.62%), *An. indefi nitus* (0.81%) and *An. maculatus* (0.81%). Characteristics of breeding sites in Mandor village were larvae of An. maculatus (11.11%), An. subpictus (3.70%), and An. vagus (85.18%). There is di erent species were found at breeding sites with di erent environmental characteristics in both high and low malaria cases in Landak District, West Kalimantan Province.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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The Effects of N-Acetylcysteine as Adjuvant Therapy To Reduce TNF-A Level And Increase SPO_2/FIO_2 Ratio In Improving Hypoxemia In COVID-19 Patients

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ABSTRACT

Tumor Necrosis Factor Alpha (TNF-α) is a pro-inflammatory cytokine that plays a crucial role in COVID-19 disease progression. N-acetylcysteine (NAC) works throughout several GSH-mediated mechanisms and is known to eliminate oxidative stress in acute respiratory distress syndrome (ARDS) in COVID-19. This study aims to analyze the e ect of the N-Acetylcysteine as Adjuvant Therapy to reduce TNF-α levels and Increase SpO_2/FiO_2 ratio in Improving hypoxemia in COVID-19 Patients. This is a quasi-experimental, non-equivalent control group design study. There were 91 subjects selected using non-random sampling, which consisted of 75 patients in the NAC group and 16 patients in the control group. The TNF-α level was measured using the ELISA method, and SpO_2/FiO_2 ratio was calculated on day 1 (on admission) and day eight after NAC 5000mg/ 72 hours was given. Statistical analysis was conducted using Wilcoxon and Mann-Whitney U Test. There is a significant decrease in TNF-α level in the treatment group (median 1.49±5.22) (p=0.016) compared with the control group (median 1.64±1.99) (p=0.005). The Median SpO_2/FiO_2 ratio on day 1 is 163.70±69.64 in the control group and 121.49±40.41 in the treatment group (p=0.005). The Median SpO_2/FiO_2 ratio on day 8 is 249.69±132.26 in the control group and 151.29±59.18 in the treatment group (p=0.001). There is a positive correlation between serum TNF-α level and SpO_2/FiO_2 ratio after administration of adjuvant therapy NAC (r=0.240, p=0.038). There is a positive correlation and significant decrease of serum TNF-α and SpO_2/FiO_2 ratio after adjuvant NAC therapy, which improves hypoxemia in COVID-19 patients.

Keywords: *N-acetylcysteine, TNF-α, SpO_2/FiO_2 ratio*

ABSTRAK

Tumor Necrosis Factor Alpha (TNF-α) merupakan sitokin proinflamasi yang berperan penting dalam perkembangan penyakit COVID-19. N-acetylcysteine (NAC) bekerja melalui beberapa mekanisme yang dimediasi GSH dan diketahui menghilangkan stres oksidatif pada acute respiratory distress syndrome (ARDS) pada COVID-19. Penelitian ini bertujuan untuk menganalisis pengaruh NAC sebagai terapi Adjuvant untuk menurunkan kadar TNF-α dan Meningkatkan rasio SpO_2/FiO_2 dalam Memperbaiki Hipoksemia pada Pasien COVID-19. Penelitian ini merupakan quasi-experimental, non-equivalent control group designed study. Subyek yang dipilih sebanyak 91 orang dengan non random sampling, yang terdiri dari 75 pasien pada kelompok NAC dan 16 pasien pada kelompok kontrol. Kadar TNF-α diukur menggunakan metode ELISA dan Rasio SpO2/FiO2 diukur pada hari ke-1 (saat masuk) dan hari ke-8 setelah pemberian NAC 5000mg/72 jam. Analisis statistik dilakukan dengan menggunakan Wilcoxon dan Mann-Whitney U Test. Terdapat penurunan kadar TNF-α yang signifikan pada kelompok perlakuan (median 1,49±5,22) (p=0,016) dibandingkan dengan kelompok kontrol (median 1,64±1,99) (p=0,005). Median Rasio SpO_2/FiO_2 pada hari 1 adalah 163,70±69,64 pada kelompok kontrol dan 121,49±40,41 pada kelompok perlakuan (p=0,005). Median rasio SpO_2/FiO_2 pada hari ke-8 adalah 249,69±132,26

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pada kelompok kontrol dan 151,29±59,18 pada kelompok perlakuan (p=0,001). Terdapat hubungan positif antara kadar TNF-α serum dengan rasio SpO_2/FiO^2 setelah pemberian terapi adjuvant NAC (r=0,240, p=0,038). Terdapat hubungan positif dan penurunan signifikan kadar TNF-α dan peningkatan rasio SpO_2/FiO_2 setelah terapi adjuvant NAC dalam memperbaiki hipoksemia pasien COVID-19.

Kata kunci: *N-Acetylcysteine, TNF-α, Rasio SpO_2/FiO_2*

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INTRODUCTION

In December 2019, there was an outbreak of coronavirus infection in Wuhan, China. On Feb 11, 2020, the World Health Organization (WHO) named the virus as "severe acute respiratory syndrome-coronavirus-2" (SARS-CoV-2), and the disease caused by this virus was referred to as "coronavirus disease-2019" (COVID-19). The ongoing COVID-19 pandemic has been hurting human health and the health care system, people's lives, and the global economy.[1]

As of Jul 28, 2020, there were 16,341,920 confirmed cases worldwide and 650,805 deaths due to COVID-19, while in Indonesia, as of Jul 28, 2020, there were 102,051 confirmed cases and 4,910 tolls due to COVID-19.[2] The high mortality rate is thought to be due to a "cytokine storm"—also known as cytokine storm syndrome. Cron and Behrens defined the cytokine storm as the activation of a cytokine-producing cascade resulting from dysregulation of the host immune response to di erent triggers. These triggers can be in the form of infection, malignancy, rheumatic disorders, etc.[3] This cytokine storm is reported to be capable of causing epithelial and endothelial cell apoptosis, vascular leakage, and ultimately acute respiratory distress syndrome and death.[4]

The host immune response to SARS-CoV-2 causes an exaggerated inflammatory reaction. Several studies analyzed the cytokine profile of COVID-19 patients and reported that cytokine storms are directly associated with lung injury, multiple organ failure, and poor prognosis of COVID-19 patients. Three pro-inflammatory cytokines are essential in innate immune response, namely Interleukin (IL)-1, IL-6, and Tumor necrosis factor (TNF)-α. The cytokine storm in COVID-19 came after a sudden increase in circulating pro-inflammatory cytokines, including elevated levels of IL-1, IL-6, and TNF-α.[5] The increasing cytokines cause an influx of various immune cells, including macrophages, neutrophils, and T cells, from the circulation to the site of infection. This reaction has a destructive e ect on human tissues caused by the process of endothelial cell destabilization, vascular barrier damage, capillary damage, diffuse alveolar damage, multiple organ failure, and ultimately death.[6]

Tumor necrosis factor-α (TNF-α) is one of the overproduced pro-inflammatory cytokines. Tumor necrosis factor-α has been associated with a poor prognosis in patients with Severe Acute Respiratory Syndrome (SARS).[7] Increase of cytokines could be seen in various inflammatory conditions, including cytokine release syndrome (CRS). TNF-α serum levels elevate in COVID-19 patients, especially those with severe symptoms.[8]

Inflammation and oxidative stress are closely related. Cell exposure to either the hydroxyl radical (-OH) or superoxide radical anion (O_2 -) causes the release of pro-inflammatory cytokines. Liposaccharides (LPS) induces intracellular accumulation of reactive oxygen species (ROS) and further increase the release of IL-1β, IL-6, and TNF-α. In addition, the nuclear factor kappa B (NF-kB) pathway is also involved in the regulation of pro-inflammatory cytokines, where this process is further amplified in Glutathione (GSH) depletion.[9]

N-acetylcysteine (NAC) works through various mechanisms by GSH within the cells. NAC has been long known as a ROS-eliminating agent, especially hypochlorous acid (HOCL) and –OH. NAC inhibition against ROS that produces Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can prevent hypertension and various pathological conditions associated with inflammation, i.e., atherosclerosis.[10] NAC has also been shown to have a protective effect against ARDS. ROS plays an essential role in the pathogenesis of lung injury, and the amount of GSH in the alveolar epithelial lining is deficient in ARDS patients. N-acetylcysteine can inhibit viral replication and the expression of pro-inflammatory molecules as well. N-acetylcysteine (NAC) can inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrophils, macrophages, IL-6, IL-1β, CXCL-10, and TNF-α. [9]

SpO_2/FiO_2 ratio (S/F ratio) was widely used recently as an alternative to PaO_2/FiO_2 (P/F ratio) to assess hypoxemia in acute respiratory failure; It is preferred due to its simplicity and non-invasiveness. In addition, it has served as a useful prognostic marker for hypoxemic respiratory failure, especially in the setting of COVID-19.

Based on the mentioned theory above and suggestions from several supporting journals, the author was interested in examining the e ect of NAC adjuvant therapy to inhibit the manifestation of the SARS-CoV2 virus through several pathways—one of which is negative regulation of NF-kB—thereby reducing the secretion of pro-inflammatory cytokines such as TNF-α.

MATERIALS AND METHODS

The study was conducted using a quasi-experimental non-equivalent control group design. The time frame was between June 2020 - July 2021. The study was conducted on patients with confirmed COVID-19 treated in the Instalasi Covid Terpadu (INCOVIT) room of RSUD dr. Saiful Anwar Malang. The subjects in this study were approved to have COVID-19 by PCR, of more than 14 years of age, and were willing to participate in the study and signed an informed consent form. Subjects who died before being treated in the INCOVIT room of dr. Saiful Anwar Hospital, where pregnant and were asymptomatic or confirmed with mild cases of COVID-19, were not included in this study.

The minimum number of samples from each dependent variable is 17. Samples were obtained by consecutive sampling. Ninety-one subjects who met the inclusion and exclusion criteria were measured for their SpO_2/FiO_2 ratio and TNF-α levels on day-0 and day-8 after receiving NAC therapy. The administration of NAC was per systemic route. We gave a bottle of 5-gram NAC via syringe pump to be given continuously over 72 hours, according to the antioxidant dose of NAC found in the literature (1,200 – 1,800 mg/day). NAC infusions are available in 5 gr bottles in our setting. The safety profile of NAC is very high as we know that a known potentially lethal dose of NAC may reach up to 100 mg/kg/ day. The antioxidant dose we use in our study is relatively safe with almost no potential side e ects, with gastrointestinal side e ects being the most frequent possible side effect found in the literature**.** Samples are also divided into two groups, the control group (treated with Standard of Care (SoC) treatments including antivirals, azithromycin, vitamin C, vitamin D, and vitamin E) and the treatment group (SoC with NAC asadjuvant therapy).

Data processing and analysis are carried out using IBM SPSS software version 16.0. The subjects were divided into the groups subjected with NAC as adjuvant therapy and without. Di erences in TNF-α on day-1 and eight were analyzed by paired T-test or Wilcoxon. The comparison of TNF-α levels at day-1 and day-8 between the groups was analyzed by independent T-test or Mann Whitney. The comparison of SpO_2/FiO_2 in the group given with NAC between day-1 and day-8 was analyzed using paired T-test or Wilcoxon. The comparison of SpO_2/FiO_2 on day-1 and eight between the groups was analyzed using the independent T-test or Mann Whitney. The relationship between changes in TNF-α levels and differences in the SpO_2/FiO_2 ratio of COVID-19 patients was analyzed using the Pearson correlation test.

RESULT AND DISCUSSION

This study was carried out from June 2020 to July 2021 in the INCOVIT room of dr. Saiful Anwar hospital, Malang. Ninety-one subjects who met the inclusion and exclusion criteria and were willing to participate in the study by signing an informed consent were found (see in Table 1).

Table 1. Demographic Profile of Research Subjects

Variable and category	NAC group (n=75)	NAC group (n=16)	P value
Age (mean ± SD, median (min-max)	52.31±11.52 56,00 (24 -76)	53.12±11.18 52,50 (25 – 69)	0.795
Sex			0.225
- Male	44 (58,7%)	12 (75%)	
- Female	31 (41,2%)	4 (25%)	
Severity			0.526
- Moderate	33 (44,0%)	3 (18,8%)	
- Severe	21 (28,0%)	11 (68,8%)	
- Critically ill	21 (28,0%)	2 (12,5%)	
Complaints			
- SOB	61 (81,3%)	16 (100%)	0.062
- Cough	60 (80%)	16 (100%)	0.052
- Fever	53 (70,6%)	16 (100%)	0.013
- Anosmia/ Ageusia	15 (20%)	10 (62,5%)	0.001
- GI disturbance	42 (56%)	10 (62,5%)	0.635
Smoker			0.253
- Yes	35 (46,67%)	10 (62,5%)	
- No	41 (54,67%)	6 (37,5%)	
Comorbidities			0.833
- Yes	35 (46,67%)	7 (43,75%)	
- No	41 (54,67%)	9 (56,25%)	
Outcome			0.369
- Recovery	64 (85,3%)	15 (93,8%)	
- Death	11 (14,7%)	1 (6,3%)	

There are 91 subjects—16 in the group not given with NAC and 75 in the group shared with. The demographic characteristics of the two groups can be seen in Table 1. The median age of patients in the NAC group was 56.00 years; this was not much di erent from the median without NAC, which was 52.50 years. Both groups were dominated by males—58.7% in the NAC group and 75% in the non-NAC group. The highest proportion of disease severity in the NAC group was moderate (58.7%), while in the non-NAC group, it was severe (68.8%). Only 81.3% of patients in the NAC group experienced shortness of breath, while those in the non-NAC group all experienced shortness of breath (100%). 46.67% of subjects in the NAC group had a history of smoking, while 62.5% in the non-NAC group had. We also noted the comorbidities on our samples, including hypertension, diabetes mellitus, asthma, chronic obstructive pulmonary disease (COPD), and cerebrovascular diseases. The distribution of patients with comorbidities in the two groups was similar and balanced (46.67% in the NAC group and 43.75% in the non-NAC group). The most significant outcome in both groups was the proportion of patients who recovered (85.3% in the NAC group and 93.8% in the non-NAC group). The NAC group's treatment length was 13.56 days, similar to the group without NAC, which was 13.87 days.

Table 2. Comparison of TNF-α levels in D1 and D8

Variable	D1	D8	p-value
TNF-α with NAC (pg/ml)	Median: 5.196 (0.97 – 16.29)	Median: 3.346 (0.26 – 17.37)	0.041
TNF-α without NAC (pg/ml)	Mean: 5.13±0.79	Mean: 6.78±2.39	0.005

From Table 2, it can be seen that of 75 subjects in the NAC group, the median value of TNF-α was 5.196 pg/ml on the first day and 3,346 pg/ml on the 8th. The Wilcoxon test resulted in a p-value of 0.041 ($p<0.05$), thus, it can be concluded that there was a significant di erence in TNF-α of the NAC group between D1 and D8, in which on D8, after administration of NAC as adjuvant therapy, TNF-α showed a median decrease of 1.85 pg/ml.

Of the 16 subjects in the group without NAC, the average of TNF-α on the first day was 5.13 pg/ml, and on the 8th day, the standard became 6.78 pg/ml. The Wilcoxon test resulted in a p-value of 0.005 ($p<0.05$), thus, indicating a significant difference of TNF-α between D1 and D8 in the non-NAC group, in which on D8, after administration of NAC as adjuvant therapy, TNF-α increased by 1.64 pg/ml.

Table 3. Comparison of TNF-α levels on D1 and D8 between the group with NAC and the group without

Variable	Non-NAC group	NAC group	P-value
TNF-α (pg/ml) on day-1 Median (min-max)	5.185 (3.65-6.40)	5.196 (0.97 – 16.29)	0.934
TNF-α (pg/ml) on day-8 Median (min-max)	5.986 (4.61-14.6)	3.346 (0.26 – 17.37)	< 0.001

Table 3 showed that, on the first day, the TNF-α levels of 91 subjects had a p-value of 0.934 (p>0.05), thus, indicating no significant di erence between the group with NAC and the group without. Furthermore, there was only a slight di erence in the TNF-α levels between the two groups on the first day (0.011 pg/ml), thus, making the difference not statistically significant.

As for the TNF-α levels on the 8th day, of 91 subjects, the p-value was 0.000 (p <0.05), thus, indicating a significant difference between the group with NAC and the group without. The difference in TNF-α levels between the two groups on day-8 was 2.64 pg/ml, thus, indicating that the TNF-α levels in the group with NAC were lower than TNF-α levels in the group without.

Table 4. Comparison of SpO2/FiO2 levels between D1 and D8

Variable	D1	D8	P-value
SpO2/FiO with NAC (unit)	Median : 108.89 (43.33 – 272.22)	Median : 138 (70.56-342.86)	<0.001
SpO2/FiO2 without NAC (unit)	Mean : 163.70±69.64	Mean : 249.69±132.26	0.001

As can be seen in Table 4, of 75 subjects in the NAC group, on the fi rst day, the median value of SpO_2/FiO_2 was 108.89 units, and on the eighth day, the median value of SpO_2/FiO_2 became 138.0 units. The Wilcoxon test obtained a p-value of 0.000 (p<0.05), thus, indicating a signifi cant di erence in the SpO_2/FiO_2 of the group with NAC between D1 and D8, in which, on D8, after adjuvant therapy, SpO_2/FiO_2 increased by 29.11 units, see Table 4.

Of the 16 subjects in the non-NAC group, on the first day, the average SpO_2/FiO_2 value was 163.70 units, and on the 8th day, the average SpO2/ FiO2 value became 249.69 units. The Wilcoxon test obtained a p-value of 0.001 (p<0.05), thus, indicating a significant di erence in the SpO_2/ FiO_2 of the group without NAC between D1 and D8, in which on H8, after adjuvant therapy, SpO_2/ FiO_2 increased by 85.99 units.

Table 5. Comparison of SpO_2/FiO_2 value between D1 and D8 between the group with NAC and the group without

Variable	non-NAC group	NAC group	P-value
SpO2/FiO2 (unit) on D1 median (min-max)	138 (70,56-342,86)	108,89 (43,33-272,22)	0.005
SpO2/FiO2 (unit) on D8 median (min-max)	193,75 (108,11-461,43)	141 (96,5-350)	0.001

The SpO_2/FiO_2 on the first day of 91 subjects showed a p-value of 0.005 (p<0.05), thus, indicating a significant di erence in SpO2/FiO2 on the first day between the group with NAC and the group without, with a di erence in SpO2/FiO2 between the two groups valuing -29.11 units, thus, indicating that SpO_2/FiO_2 on the first day in the group with NAC was lower than SpO_2/FiO_2 in the group without, see Table 5.

As for the SpO_2/FiO_2 test on the eighth day, of 91 subjects, a p-value of 0.001 (p<0.05) was obtained, thus, indicating a significant di erence in SpO_2/FiO_2 on day-8 between the group with NAC and the group without, with a di erence of SpO_2/FiO_2 between the two groups valuing -98.40 units, thus, indicating that SpO_2/FiO_2 on day-8 in the group with NAC was lower than SpO_2/FiO_2 in the group without.

Table 6. Pearson correlation test between the changes of TNF-α (pg/mL) and the changes of SpO_2/FiO_2 ratio (D8-D1)

	Delta Ratio SpO2/ FiO2 NAC group	Delta Ratio SpO2/FiO2 non-NAC group
Delta TNF-α (pg/mL) NAC group (H8-H1)	r = 0,240 p = 0,038 n = 75	r = 0,292 p = 0,272 n = 16

The Pearson correlation test for the relationship between changes in TNF-α levels and SpO_2/FiO_2 ratio of COVID-19 patients after administration of NAC as adjuvant therapy resulted in the correlation coefficient value of 0.240 with a significance value (p) of 0.038 ($p<0.05$), thus, indicating a positive and significant correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio. A positive coe cient value could be interpreted as the higher the change in TNF-α levels in COVID-19 patients after administration of adjuvant therapy, the higher the SpO_2/FiO_2 ratio will be. On the other hand, the lower the change in TNF-α levels in COVID-19 patients after adjuvant treatment, the lower the SpO_2/FiO_2 ratio, see Table 6.

The results of this test prove that the hypothesis of the correlation test between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients after administration of adjuvant NAC therapy is true. There is a positive correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio of COVID-19 patients after administration of adjuvant NAC therapy.

The relationship between changes in TNF-α levels and SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy shows a correlation coe cient value of -0.292 with a significance value (p) of 0.272 ($p>0.05$), thus, indicating neither positive nor significant correlation between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant therapy. In other words, the level of change in TNF-α levels in COVID-19 patients in the group without adjuvant NAC therapy did not a ect the level of change in the SpO_2/FiO_2 ratio.

The results of this test prove that the hypothesis of the correlation test between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy that there is a positive correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio of COVID-19 patients who did not receive adjuvant NAC therapy is not valid, because the correlation coe cient was negative and not significant.

The characteristics of the groups with NAC and those without did not di er by much in age. The median age in the group with NAC was 56.00 years, while in the group without was 52.50 years. NAC works as an antioxidant and anti-inflammatory.[11] The occurrence of inflammation and oxidation increases with age[12], as age may be one of many factors a ecting clinical outcomes in COVID-19 patients. However, in this study, the median age did not di er much. Thus, the baseline for the two groups was the same.

Both groups in this study were dominated by the male gender—58.7% in the NAC group and 75% in the non-NAC group. This result should be considered as it can lead to confusion in data analysis. Gender is one of the predictors of COVID-19 outcome, where a male is associated with a worse prognosis compared to a female.[13]

There were some di erences in the clinical profile between the two groups in this study. First, the highest proportion of disease severity in the NAC group was moderate (58.7%), while in the non-NAC group, the highest proportion was severe (68.8%). Second, only 81.3% of the subjects in the NAC group experienced shortness of breath, while in the group without, all subjects experienced symptoms of shortness of breath (100%). Third, the most significant outcome in both groups was the proportion of patients who recovered (85.3% in the NAC group and 93.8% in the non-NAC group). Di erences in these clinical conditions can be confounding factors, and thus, need to be considered in interpreting the results.

The distribution of subjects with comorbidities was similar in the two groups (46.67% in the NAC group and 43.75% in the non-NAC group). In addition, the length of treatment for the NAC group was 13.56 days, comparable to the group without NAC, which was 13.87 days. These two indicators do not di er much in the two groups; thus, comorbid factors and length of treatment are not considered things that can confound the results.

TNF-α in the group with NAC showed a reduction of 1.49 pg/ml and a statistically significant di erence. TNF-α in the NAC group was lower in D8 than D1 ($p=0.041 <0.05$). In the group without NAC, TNF-α increased on the eight

day compared to the first day (p=0.005 <0.05). This is by the hypothesis that NAC administration as an adjuvant to COVID-19 therapy can reduce TNF-α.

TNF-α is one of the pro-inflammatory cytokines that worsens the outcome of COVID-19 infection. TNF-α is produced by cells in the immune system, such as macrophages, dendritic cells, and T cells, during inflammation.[14] In severe COVID-19 infection, TNF-α is produced in excess, causing an increase in the systemic inflammatory response that results in tissue damage. One of the functions of TNF-α is to increase the recruitment of phagocytic cells and increase the autolysis reaction of cells.[15] TNF-α is associated with acute lung injury and predicts prognosis.[5]

Oxidative stress and inflammation are closely related to each other. Cell exposure to either the hydroxyl radical (-OH) or superoxide radical anion (O2-) causes the release of pro-inflammatory cytokines. Liposaccharides (LPS) induces intracellular accumulation of reactive oxygen species (ROS) and further increase the release of IL-1β, IL-6, and TNF-α. In addition, the nuclear factor kappa B (NF-kB) pathway is also involved in the regulation of pro-inflammatory cytokines, where this process is further amplified under the conditions of Glutathione (GSH) depletion.[9]

N-acetylcysteine has recently been suggested as adjuvant therapy in standard treatment for SARS-CoV-2 infection. NAC benefits by increasing glutathione synthesis, enhancing immune function, and modulating the inflammatory response. There are two therapeutic mechanisms of action of NAC: 1) Mucolytic e ect caused by the free sulfhydryl groups, which reduces disulfide bonds in the mucus glycoprotein matrix, thereby reducing mucus viscosity; 2) Antioxidant effect caused by direct interaction with free radicals, as well as indirect e ect as cysteine precursor required for glutathione biosynthesis, and replenishment of thiol pool which is central to redox regulation and control.[16]

N-acetylcysteine (NAC) acts through various mechanisms mediated intracellularly by GSH. NAC has long been known as a ROS-eliminating agent, especially hypochlorous acid (HOCL) and –OH. NAC inhibition against ROS that produces the oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) can prevent hypertension and various pathological conditions associated with inflammation, such as atherosclerosis.[10] In addition, NAC has also been proven for its protective e ect against ARDS, as ROS plays an essential role in the pathogenesis of lung injury, and the alveolar epithelial lining of ARDS patients is deficient in GSH. N-acetylcysteine can also inhibit viral replication and the expression of pro-inflammatory molecules. N-acetylcysteine (NAC) has been proven to inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrophil macrophages, IL-6, IL-1β, CXCL-10, and TNF-α.[9]

Previous studies found that NAC administration significantly decreased CRP and NEWS2 scale levels. The duration of hospitalization was also considerably shorter in the NAC group. However, all other clinical outcomes—transfer to ICU, need for non-invasive or invasive mechanical ventilation, and 28-day mortality—do not di er between the two groups.[17]

Several studies have also examined the e cacy of NAC in hospitalized patients with COVID-19. In respirator-dependent patients, intravenous administration of NAC results in clinical improvement and reduction of CRP and Ferritin.[18] Another study by Alamdari et al. had shown that administration of NAC in combination with high doses of methylene blue and vitamin C as a last resort therapy resulted in a significant recovery and clinical response in four out of five critically ill patients with COVID-19.[19] At the same time, de Alencar et al. suggested that the administration of high doses of NAC does not a ect the evolution of severe COVID-19.[20]

In both groups, the SpO_2/FiO_2 ratio increased significantly on the eighth day compared to the first day. However, when comparing the increase of SpO_2/FiO_2 ratio between the two groups, it was found that the group without adjuvant therapy had a higher increase in the SpO_2/FiO_2 ratio. This finding was by another study, which found that the administration of NAC could significantly increase the SpO_2FiO_2 ratio.[17]

Most of the subjects in the non-NAC group had severe degrees of illness. However, the outcome in the group without NAC was also better, in which 93.8% recovered, while in the NAC group, only 85.3%. This causes the increase in the SpO_2/FiO_2 ratio on the 8th day in the group without NAC to be higher than the group with NAC.

A commensurate baseline is needed to obtain unbiased results, where the clinical conditions are similar for the groups given NAC and the groups not. Therefore, further research using a larger sample size or a placebo-controlled experimental study (randomized controlled trial) is necessary.

Changes in TNF-α levels and alterations in SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy showed a positive correlation. On the other hand, in the group that did not receive adjuvant NAC therapy, the correlation coe cient was negative and not significant.

This finding is by the previous studies, in which it was found that the administration of NAC can increase the SpO_2/FiO_2 ratio.[16] The conclusions of this study prove that there is the involvement of TNF-α in the mechanism of action of NAC. NAC interferes with the NLRP3 inflammasome pathway by suppressing the expression of NLRP3 and caspase-1 activation mRNA. NAC decreased IL1β, IL18, IL6 and TNF-α in vitro. NAC inhibits downstream activity post-TNF-α receptor activation, and under oxidative stress, NAC inhibits TNF-α and IL-6 gene expression.[11]

CONCLUSION

There was a significant decrease in TNF-α levels after NAC administration as adjuvants. In addition, there was also a substantial increase in the SpO_2FiO_2 ratio after administration of adjuvant NAC therapy in patients with COVID-19. However, there was no change in SpO_2/FiO_2 in the subjects given adjuvant NAC therapy and not. This study also showed a significant positive correlation between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio in COVID-19 patients after administration of adjuvant NAC therapy. On the other hand, there was no positive correlation between changes in TNF-αlevels and diferences in the SpO_2/FiO_2 ratio in COVID-19 patients who did not receive adjuvant NAC therapy.

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CONFLIC OF INTEREST

The authors declare that they have no conflict of interest.

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