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Comparison of Neutrophile-to-Lymphocyte Ratio Between Dengue Haemorrhagic Fever and Dengue Shock Syndrome in Pediatric Patients at Anutapura Hospital

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LITERATURE REVIEW

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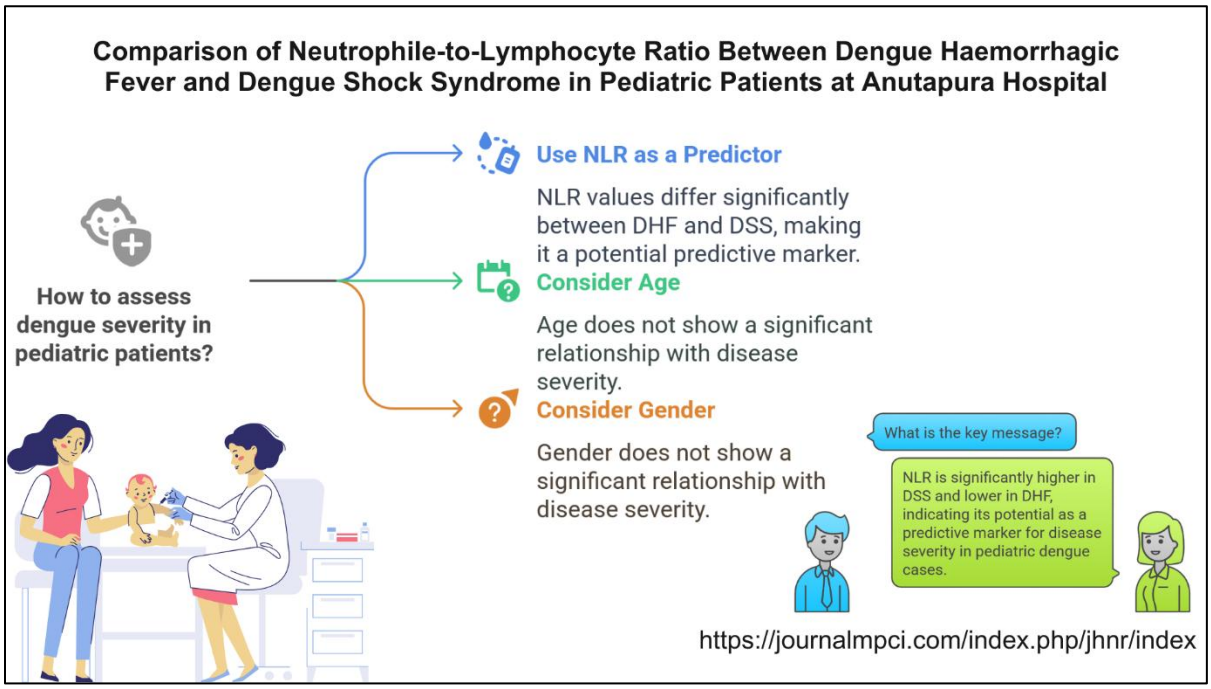
ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an acute viral infectious disease that attacks the body. DHF is divided into 4 grades (I, II, III, IV), in which degrees 3 and 4 are also known as Dengue Shock Syndrome (DSS). When the disease severity is detected too late, it can be fatal. Therefore, a predictor or inflammatory marker is needed to detect and predict this. In this study, the inflammatory marker assessed is the NLR value. This study compares the neutrophil-lymphocyte ratio and the incidence of Dengue Hemorrhagic Fever and Dengue Shock Syndrome in pediatric patients at the Anutapura Regional Hospital, Central Sulawesi Province. This study uses an observational analytical method with a cross-sectional design using secondary data from medical records at the Anutapura Regional Hospital, Palu, in 2021-2023. The sample included is patients with a diagnosis of DHF, totaling 100 samples. The sampling technique used is random sampling. Based on the Mann-Whitney test, a significant difference (p = 0.001) was found in the NLR value between DHF and DSS. The mean NLR in DHF was lower (1.28 (±0.98 SD)) than in DSS (2.07 (±1.59 SD)). There was no significant relationship between age (p=0.217) and gender (p=0.597) in the DHF and DSS groups. It is then concluded that there is a significant difference in NLR values between DHF and DSS, where NLR values are lower in DHF and higher in DSS.

Key Messages:

- The Neutrophil-Lymphocyte Ratio (NLR) is significantly elevated in pediatric patients with Dengue Shock Syndrome (DSS) compared to those with less severe Dengue Hemorrhagic Fever (DHF), suggesting NLR's potential utility as an inflammatory marker for assessing dengue disease severity in this population

GRAPHICAL ABSTRACT



INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a viral infection with sudden onset, often leading to severe complications. DHF has 4 degrees of severity with different manifestations, in which degrees 3 and 4 are also known as Dengue Shock Syndrome (DSS). Symptoms include high fever for 2-7 days, accompanied by bleeding, low platelets (thrombocytopenia), and plasma leakage (increased hematocrit, ascites, pleural effusion, hypoalbuminemia). Other symptoms such as headache, muscle and bone pain, skin rash, and pain behind the eyeballs can also occur, although they are not always present (1). The pathogenesis of DHF involves increased capillary permeability, which can lead to plasma leakage and hypovolemic shock (2). From 2000 to 2019, the World Health Organization documented a tenfold increase in reported cases worldwide from 500,000 to 5.2 million. 2019 marked an unprecedented peak, with reported cases spread across 129 countries (3). The incidence rate (IR) in Indonesia in 2023 has not reached the national target of dengue fever incidence rate ≤10 per 100,000 population, with the actual rate being 41.4 per 100,000. This figure has decreased compared to 2022, with an IR of 52.1 (4).

In Central Sulawesi in 2023, the dengue fever incidence rate was 56.4 per 100,000 population, indicating that it had not reached the national target (4), this has increased significantly compared to 2021 with an IR of 21.5 per 100,000 population (5). Likewise, data from Anutapura Hospital, which is one of the main referral hospitals in Central Sulawesi Province for handling dengue patients. Based on the 2017 Guidelines for Prevention and Control of Dengue Hemorrhagic Fever, dengue diagnosis is established through clinical and laboratory criteria with serological examination of anti-dengue or dengue non-structural antigen-1 (NS1) or PCR.[1] Currently, most dengue diagnoses are based on clinical criteria. In case studies in Yogyakarta City in the period of November-December 2016, of the 230 cases reported, only around 45% of reported dengue cases were accompanied by laboratory confirmation of either dengue antigen or anti-dengue antibody. Some suspected dengue cases are examined using RDT (Rapid Diagnostic Test), and not all health centers in dengue-endemic areas provide this rapid diagnostic tool. In addition to RDT, a tourniquet test to detect capillary fragility is recommended as a simple and inexpensive method to help diagnose dengue (6). In cases of Dengue, knowing the severity of the diagnosis and late management can have serious consequences for the patient. A complete blood count that is inexpensive, easy to perform, and available at various health facilities can help overcome this problem. Specific blood components can provide an overview of the development of the disease and the patient's health condition (7).

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Neutrophil-to-Lymphocyte Ratio (NLR), can indicate systemic inflammation and disease severity in various conditions, including viral infections (8). Studies specifically on dengue have often shown a correlation between elevated NLR values and increased disease severity, potentially predicting outcomes like plasma leakage or the development of DSS(9). In 2011, the World Health Organization established diagnostic criteria for Dengue based on leukocyte, platelet, and hematocrit levels. These criteria help determine the diagnosis and severity of the infection (10). Leukocytes play an important role in fighting viruses through phagocytosis, antibody formation, and lymphocyte activation to destroy or deactivate viruses. Neutrophils and lymphocytes, representing 80% of leukocytes, play a crucial role in inflammation and infection. Therefore, the immune response can be assessed by comparing the levels of these two cells (7). The Neutrophil-Lymphocyte Ratio (NLR), derived from routine blood tests, is a simple, cost-effective index that can help predict dengue infection and assess severity earlier. This study aims to contribute to this understanding by specifically comparing NLR values between DHF and the more severe DSS form within a pediatric population at Anutapura Hospital, potentially identifying patterns relevant for early risk assessment in this setting.

METHODS

This study uses an observational analytical method with a cross-sectional design using secondary data from medical records at the Anutapura Palu Regional Hospital in 2021-2023. The total population identified from the hospital medical records during this period who were diagnosed with DHF or DSS was 509 patients. The minimum required sample size was calculated using Slovin's formula with a margin of error (e) assumed to be 0.1 (10%), based on this population size (N=509):

$$n = \frac{509}{1 + 509(0,1)^2}$$
$$= \frac{509}{6,09}, = 83,57$$

Therefore, the minimum sample in this study was adjusted to 84 samples. The sample included 100 patients diagnosed with Dengue Hemorrhagic Fever or Dengue Shock Syndrome who met the criteria. This study used a random sampling technique, which is a sampling technique in which each member of the population has an equal chance of being selected as a sample.

The sample in this study has inclusion and exclusion criteria. Inclusion Criteria: 1) Patients aged 1-18 years; 2) Patients diagnosed with DHF/DHF and DSS; 3) Patients who are hospitalized in 2021-2023. Exclusion Criteria: 1) DHF patients whose medical records are incomplete; 2) Patients who suffer from complications of other diseases.

Age

The participant's age was defined as their age in years at the time of hospital admission. This information was obtained through observation of data recorded within the patient's medical records. For analysis, age was treated as an ordinal variable and categorized into three groups: Toddler (1-4 years), Child (5-9 years), and Adolescent (10-18 years).

Neutrophil Count

Neutrophil count was operationally defined as the percentage of neutrophils relative to the total white blood cell count, obtained from the complete blood count (CBC) laboratory results documented in the patient's medical record. It served as an indicator related to the inflammatory response. This variable was measured on an ordinal scale based on predefined reference ranges: Low (<54%), Normal (54-62%), and High (>62%).

Lymphocyte Count

Lymphocyte count represented the percentage of lymphocytes relative to the total white blood cell count, as reported in the CBC results found in the patient's medical record. This variable reflects aspects of the immune response relevant to viral infections. Data were observed from the medical records and categorized using an ordinal scale: Low (<25%), Normal (25-33%), and High (>33%).

Neutrophil-to-Lymphocyte Ratio (NLR)

The Neutrophil-to-Lymphocyte Ratio (NLR) was operationally defined as a calculated index derived by dividing the neutrophil percentage by the lymphocyte percentage obtained from the same CBC test

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documented in the patient's medical record. The resulting value was categorized using an ordinal scale based on a predetermined cut-off for this study: Normal (<3.13) and High (≥3.13).

Dengue Haemorrhagic Fever (DHF)

Dengue Haemorrhagic Fever (DHF) was defined based on the clinical diagnosis recorded in the patient's medical record by the attending physician, corresponding to WHO dengue severity classification Grades 1 or 2 as applied at the study hospital. This variable represents the non-severe dengue outcome group in the primary analysis comparing DHF and DSS, identified through observation of the final diagnosis in the medical records.

Dengue Shock Syndrome (DSS)

Dengue Shock Syndrome (DSS) was defined according to the clinical diagnosis documented in the patient's medical record, indicating severe dengue corresponding to WHO dengue severity classification Grades 3 or 4, characterized by evidence of circulatory failure. Identification was based on observation of the final diagnosis within the medical records, representing the severe dengue outcome group for comparison against DHF.

The research instrument is a medical record to know the incidence of Dengue Hemorrhagic Fever at the Anutapura Regional Hospital, Central Sulawesi Province for 2021-2023. NLR levels are measured using complete blood tests, calculating neutrophil results divided by lymphocyte results. Bivariate analysis was performed using the Mann-Whitney test with the SPSS program.

CODE OF HEALTH ETHICS

Ethical clearance was granted by the Medical and Health Research Ethics Committee, Faculty of Medicine, Tadualko University, with Number: 5752/UN28.10/KL/2024.

RESULTS

Respondent Characteristics

Table 1 show that the distribution of the number of patients based on age, there were 25 patients in the toddler category (1-4 years), 26 patients in the child category (5-9 years) and 49 patients in the adolescent category of age (10-18 years). Based on the distribution of gender, there were 47 male patients and 53 female patients.

Table 1 Demographic Characteristics of Study Participants

Characteristic		n	%
Age	1-4 years old	25	25
	5-9 years old	26	26
	10-18 years old	49	49
Gender	Male	47	47
	Female	53	53
Total		100	100%

The distribution of neutrophil values showed that 71 participants had low levels, 13 had normal levels, and 16 had high levels (Table 2). Table 3 shows that a cross-tabulation examining the distribution of categorized neutrophil and lymphocyte percentages across patients diagnosed with Dengue Haemorrhagic Fever (DHF, n=83) and Dengue Shock Syndrome (DSS, n=17). Regarding neutrophil levels, most patients in both groups exhibited low neutrophil counts (<54%). Specifically, 61 of 83 DHF patients (73.5% of DHF cases) and 10 of 17 DSS patients (58.8% of DSS cases) fell into this category. However, a notable difference appears in the high neutrophil category (>62%); this was observed in only 10 DHF patients (12.0%) but in 6 DSS patients (35.3%). Normal neutrophil levels (54-62%) were infrequent in both groups, particularly in DSS (1 patient, 5.9%). For lymphocyte levels, DHF patients predominantly showed high lymphocyte counts (>33%), with 55 out of 83 cases (66.3%) in this category. In contrast, the distribution among DSS patients was more varied: high lymphocytes were less common (7 out of 17 cases,

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41.2%), while low (<25%) and normal (25-33%) lymphocyte counts were equally represented (5 cases each, 29.4% each).

Table 2 Neutrophil and Lymphocyte values in DHF and DSS patients

Neutrophil and Lymphocyte	n	%
Neutrophil Value		
Low (<54%)	71	71
Normal (54-62%)	13	13
High (>62%)	16	16
Lymphocyte Value		
Low (<25%)	14	14
Normal (25-33%)	24	24
High (>33%)	62	62
Total	**100**	**100**

Table 3 Cross-tabulation of neutrophil, lymphocyte, and diagnosis values

Diagnosis	Neutrophils						Lymphocytes							
	Low (<54%)		Normal (54-62%)		High (>62%)		Low (<25%)		Normal (25-33%)		High (>33%)		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
DHF	61	61.0	12	12.0	10	10.0	9	9.0	19	19.0	55	55.0	83	83.0
DSS	10	10.0	1	1.0	6	6.0	5	5.0	5	5.0	7	7.0	17	17.0
Total	71	71.0	13	13.0	2	2.0	2	2.0	64	64.0	34	34.0	100	100

Table 4 summarizes the comparison of the Neutrophil-to-Lymphocyte Ratio (NLR) between patients diagnosed with Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). The analysis reveals a statistically significant difference in the mean NLR values between the two groups (p = 0.001). Patients with DSS exhibited a significantly higher mean NLR (Mean ± SD = 2.07±1.59) compared to patients with DHF (Mean ± SD = 1.28±0.98). Furthermore, when NLR was categorized into 'Normal' and 'High' levels (based on the study's defined cut-off, likely <3.13 as Normal from the operational definitions), a significant association was found between these categories and the diagnosis groups (p = 0.001). Although the table displays the distribution across groups, calculating within-group proportions shows that a 'High' NLR was observed more frequently in DSS patients (4 out of 17, or 23.5%) than in DHF patients (2 out of 83, or 2.4%). Conversely, 'Normal' NLR was observed in most DHF patients (81 out of 83, or 97.6%) and many DSS patients (13 out of 17, or 76.5%).

Table 4 Comparison of NLR categories between DHF and DSS

Parameter		DHF	DSS	p-value
NLR	Normal	81 (86,2%)	13 (13,8%)	0,001
	High	2 (33,3%)	4 (66,7%)	
	Mean ±	1,28 ± 0,98	2,07 ± 1,59	0,001

Table 5 presents an analysis investigating the relationship between potential confounding variables, specifically age group and gender, and the clinical diagnosis groups (Dengue Haemorrhagic Fever - DHF vs. Dengue Shock Syndrome - DSS). The distribution of patients across different age categories (1-4 years, 5-9 years, 10-18 years) was compared between the DHF and DSS groups. Although there were some variations in the percentage distribution (e.g., 10-18 years constituted 51.8% of DHF cases vs. 35.3% of DSS cases), the statistical test yielded a p-value of 0.189. As this value is greater than the conventional significance level of 0.05, there was no statistically significant difference in this cohort's age distribution between the DHF and DSS groups. Similarly, the distribution of gender was examined. Females represented a slight majority in both groups (51.8% in DHF, 58.8% in DSS). The comparison between the DHF and DSS

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groups regarding gender distribution resulted in a p-value of 0.597. This p-value, being well above 0.05, signifies that no statistically significant association was found between gender and the likelihood of having DHF versus DSS.

Table 5 Relationship of confounding variables with DHF and DSS groups

Confounding variable	Group				p
	DHF		DSS		
	n	(%)	n	(%)	
Age					
1-4 years old	19	(22,9)	6	(35,3)	0,189*
5-9 years old	21	(25,3)	5	(29,4)	
10-18 years old	43	(51,8)	6	(35,3)	
Gender					
Male	40	(48,2)	7	(41,2)	0,597
Female	43	(51,8)	10	(58,8)	

DISCUSSION

This study was conducted in December 2024 at the Anutapura Regional Hospital, Central Sulawesi Province. This study began with the administration of administrative files at the Anutapura Regional Hospital, Central Sulawesi Province, after which the data collection process was carried out in the medical records room, and then the research variables were recorded. The initial data were obtained from 509 patients diagnosed with DHF or DSS. However, the researcher has not been able to determine the exact number of patients diagnosed with DHF and how many were diagnosed with DSS due to limited access and permits to access physical medical records directly. The authors could only access medical data recorded in the Anutapura digital medical record system. After determining the population, the researcher determined the minimum number of samples using the Slovin formula, which resulted in 84 patients. The results of the search for medical records of DHF and DSS patients obtained 63 medical records in 2023, 22 medical records in 2022, and 15 medical records in 2021, with the number of samples that the researcher has calculated and adjusted to 100 samples, which met the inclusion and exclusion criteria. The samples from medical record data that fall into the exclusion criteria are samples diagnosed with dengue fever but have comorbidities, including pneumonia, bronchitis, dyspepsia, acute tonsillopharyngitis, and other infectious diseases that have the potential to affect the neutrophil-lymphocyte ratio.

In this study, the authors also discussed the characteristics of the subjects, specifically gender and age. This study showed no significant difference (p = 0.597) between female and male genders in the incidence of DHF and DSS because this study only took data that fell into the inclusion and exclusion criteria. This is in line with research conducted by Salsabila (2017), which stated that there was no significant relationship between gender and the incidence of SSD at RSD Dr. Soebandi Jember district, which has a p-value = 0.198 (11).

The results of this study also showed that there was no significant relationship (p = 0.189) between the age of toddlers (1-4 years), children (5-9 years), and adolescents (10-18 years) and the incidence of DHF and DSS. This is in line with the study of Salsabila (2017), which stated that there was no significant relationship between age and the incidence of DSS because the target of mosquitoes to suck blood is at all ages. This study found more patients in the age range of 10-18 years. This aligns with research conducted by Ramadani (2023), which states that the most extensive age range is 17-25 years or in adolescents. This is due to the late adolescent age group, which tends to be more productive and active and has many additional activities outside the home, so it is more at risk of being bitten by the Aedes aegypti mosquito. This aligns with research by Susanti (2022), which states that school-age children or adolescents often spend from morning to evening in a room, one of the places with the highest risk. School-age children are usually infected when they are active in the school garden and play in the afternoon. However, this is not in line with research by Permatasari (2015), which states that children aged 5 years are more

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susceptible to dengue fever because the immune response with specificity and immunological memory stored in dendritic cells and lymph glands is not yet perfect (11–14).

Based on the results of cross-tabulation between neutrophil values and patient diagnoses, it was found that more patients had low neutrophil values, namely DHF (61%) and DSS (10%). Likewise, the cross-tabulation results between lymphocyte values and patient diagnoses showed that more patients had high lymphocyte values, namely DHF (55%) and DSS (7%). It can be concluded from the cross-tabulation results that DHF and DSS patients have low neutrophil values and high lymphocytes, which dominate. This aligns with the existing theory, which states that in the early phase of dengue fever, the number of leukocytes is generally normal, with the number of neutrophils dominating. After that, in the critical phase, the number of leukocytes will decrease, namely <5000 cells per cubic millimeter, so that it affects the NLR value, namely, the number of neutrophils decreases and lymphocytes increase (6).

This is in line with Harahap's research (2015), which states that there is a decrease in the number of neutrophils in pediatric patients with dengue virus infection (15). According to Meliala (2021), Neutrophils are the body's line of defense, and the presence of immunomodulators can increase the immune response. At the beginning of a viral infection, neutrophils, which are the main components of leukocytes, will actively immigrate to the immune system and are used in tissues due to infection (16). Therefore, the number of neutrophils in the bloodstream will reflect the body's ability to maintain homeostasis in various conditions. An increase in the number of neutrophils in the blood circulation is known as neutrophilia, which can be caused by inflammation, necrosis, or acute stress, which causes a decrease in the ability of neutrophils to immigrate to tissue. The decrease in the number of neutrophils below the normal range in the blood circulation is known as neutropenia, which can be caused by viral infections and malnutrition of folic acid, copper, and vitamin B12 (17,18). Lymphocytes are one of the important components of leukocytes in the immune system that respond to antigens to form antibodies. During a viral infection, adaptive immune system cells will become active when recognizing antigens through their antigen-specific receptors. An increase in the number of lymphocytes in the blood is known as lymphocytosis, which can be caused by viral, parasitic, and bacterial infections, while a decrease in lymphocytes is caused by stress, age, nutritional intake, and physiological activity (17,19). The percentage of neutrophils will gradually decrease, accompanied by an increase in the percentage of lymphocytes and the duration of fever during dengue infection. An increase in the number of lymphocytes occurs on the 5th to 9th day since the duration of the fever and appears more often when entering the healing phase. The NLR value is also low when the number of neutrophils is low. and the higher the lymphocytes, the lower the NLR value (7,20).

According to Yuditya (2020), normal NLR levels in pediatric patients with dengue fever indicate that they are unlikely to experience significant changes in their NLR in response to dengue infection. Some cases of dengue fever have changes in NLR levels, which indicates that the disease is getting worse (18). According to Meliala (2021), NLR and platelets have significant differences, where the results obtained show that the average plasma leakage value in neutrophils and lymphocytes is higher than plasma leakage in platelets (16). According to Yuntoharjo (2018), NLR will increase systemic inflammation or severe infection, indicating that clinical evaluation can be used in patients with systemic inflammation. Neutrophils and lymphocytes constitute 80% of the total number of leukocytes and function as a response to infection, so most of the immune response can be described from the levels of these two cells. NLR examination can be used as a marker of the infection process in the body (21).

The results of the Mann-Whitney test showed a p-value of 0.001, indicating $p < 0.05$, which means there is a significant difference between the neutrophil-lymphocyte ratio (NLR) values in DHF and DSS in pediatric patients. This is in line with research conducted by Irmayanti (2017), which stated that there was a significant difference between the NLR value in the DHF grade 1 and grade 2 groups (22). This is in line with research by Cahyani (2020), which stated that there was a relationship between the number of platelets, hematocrit levels, and NLR with the duration of fever in DHF patients (20). This is in line with research by Prijanto (2023), which stated that there was a relationship between NLR and shock conditions.[7] This is in line with research by Yuntoharjo (2018), which showed that the NLR value was lower in DHF patients compared to DD (21). This is in line with research by Yuditya (2020), which stated that

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there was a significant relationship between NRL and the severity index of dengue infection in adult patients (18).

This is not in line with research by Djalilah (2022), which stated that there was no relationship between NLR values and dengue infection in children because the study had limitations, namely only having 54 samples, which were indicated as being less able to represent specific characteristics in a study and the criteria for diagnosing dengue infection found in the medical records is not based on serological examination but instead based on a doctor's diagnosis. This is also not in line with research conducted by Nusa (2015) which stated that there was no significant relationship between the neutrophil and lymphocyte ratio and dengue virus infection ($p = 0.630$) because there are several things that affect the results of the leukocyte count, namely the quality and type of equipment used and the skills of laboratory personnel who examine and the examination of the type of white blood cell count for neutrophils and lymphocytes was carried out on different days; days 2, 3, 4, 5, 6, and 7 (23,24).

The study lasted over a month and was not free from several limitations encountered when collecting medical record data at the Anutapura Regional Hospital, Central Sulawesi Province. A major limitation is that the researchers can only open digital medical records that do not have complete dates of various examination results, such as physical medical records, and physical medical records that are available in complete and well-organized form are only the ones from 2023. Furthermore, while NLR values are known to fluctuate during the course of dengue illness, this study was limited by the inability to consistently ascertain the precise timing (e.g., day of fever, febrile vs. critical phase) of the complete blood count sample used for NLR calculation for all participants due to variations in digital record completeness. This lack of standardized timing for the NLR measurement represents a limitation when comparing the findings to literature describing the dynamic changes of NLR throughout disease progression and interpreting the observed differences between DHF and DSS. The convenience obtained by researchers is that retrieving patient medical records was more straightforward because they used digital medical records on the computer. Another convenience is that the diagnostic group of this study's independent variables were readily available in the medical records. Therefore, the researchers did not have to determine the category of dengue fever grades based on WHO criteria.

CONCLUSION

There are lower neutrophil levels and high lymphocyte levels in Dengue Haemorrhagic Fever and Dengue Shock Syndrome cases at Anutapura Hospital, Central Sulawesi Province. There are more adolescents admitted with Dengue Haemorrhagic Fever, while in Dengue Shock Syndrome, there are more toddlers and children, both of which are dominated by the female gender. There is a significant difference in the neutrophil-lymphocyte ratio between Dengue Haemorrhagic Fever and Dengue Shock Syndrome; the neutrophil-lymphocyte ratio is higher in Dengue Shock Syndrome compared to Dengue Haemorrhagic Fever.

Future studies should aim to include a larger and more balanced sample size, particularly for DHF cases, to improve the reliability and generalizability of the results. In addition, the neutrophil-lymphocyte ratio can be evaluated serially, specifically from when the patient is admitted to the hospital until they reach recovery.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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