

# MONOCYTE LYMPHOCYTE RATIO IN DENGUE HEMORRHAGIC FEVER

*by* Dwi Retnoningrum

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RESEARCH

**MONOCYTE LYMPHOCYTE RATIO IN DENGUE HEMORRHAGIC  
FEVER**

*(Monocyte Lymphocyte Ratio di Dengue Hemorrhagic Fever)*

Dwi Retnoningrum, Purwanto AP

**ABSTRAK**

Dengue Hemorrhagic Fever (DHF) adalah penyakit yang disebabkan oleh virus dengue, virus yang dapat ditularkan melalui gigitan nyamuk. Dengue hemorrhagic fever merupakan salah satu penyebab kesakitan dan kematian terbanyak di dunia termasuk di Asia. Patogenesis infeksi DHF diduga melibatkan monosit dan limfosit akibat dari respons imun terhadap infeksi. Monocyte Lymphocyte Ratio (MLR) sebelumnya digunakan dalam menggambarkan respons imun di infeksi malaria, tuberkulosis dan HIV. Penelitian ini bertujuan untuk mengetahui apakah terdapat perbedaan MLR di infeksi DHF derajat ringan dan berat. Metode penelitian ini observasional analitik dengan desain potong lintang di pasien DHF di RS Dr. Kariadi Semarang masa waktu Januari-Desember 2013. Nilai MLR didapat dari perhitungan jumlah monosit dibagi jumlah limfosit dari hitung jenis leukosit. Derajat DHF ditentukan sesuai dengan patokan WHO, yaitu derajat I-II masuk dalam derajat ringan, derajat III-IV adalah derajat berat. Analisis statistik dengan Student t test. Kelompok I terdiri dari 40 pasien DHF derajat ringan dan kelompok II terdiri dari 40 pasien DHF derajat berat. Subjek terdiri dari 41 laki-laki (51,2%) dan 39 perempuan (48,8%). Rentang nilai MLR di DHF derajat ringan ditemukan dari 0,03–0,33 (median 0,11) sedangkan di DHF derajat berat dari 0,03–0,59 (median 0,16). Analisis statistik menunjukkan tidak terdapat perbedaan nilai MLR antara kelompok I (derajat ringan) dan kelompok II (derajat berat) ( $p=0,08$ ). Tidak didapatkan perbedaan nilai MLR di infeksi DHF derajat ringan dan berat.

**Kata kunci:** Dengue hemorrhagic fever, monocyte lymphocyte ratio, derajat DHF

**ABSTRACT**

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus transmitted through mosquito bites. Dengue hemorrhagic fever causes high morbidity and mortality in the world, including Asia, especially in Indonesia. The pathogenesis of DHF infection is thought to involve monocytes and lymphocytes as a result of the immune response to infection. Monocyte Lymphocyte Ratio (MLR) previously used in describing the immune response to malaria infection, tuberculosis and HIV. To determine whether there are differences between MLR in mild and severe DHF. A cross-sectional study in DHF patients at the Dr. Kariadi Hospital Semarang was done in January to December 2013. Monocyte lymphocyte ratio value was obtained from calculating the number of monocytes divided by lymphocytes from leukocyte differential count. The degree of DHF was determined in accordance to the WHO criteria, where grade I-II as considered as a mild degree and grade III-IV was considered as severe degree. Statistical analysis analyzed by Student's t test. Group I consisted of 40 patients with mild DHF and group II consisted of 40 patients with severe DHF. The subjects consisted of 41 males (51.2%) and 39 females (48.8%). Monocyte lymphocyte ratio value in mild DHF was from 0.03 to 0.33 (median 0.11), while the severe DHF from 0.03 to 0.59 (median 0.16). Statistical analysis showed there was no significant difference between the value of MLR group I (mild) and group II (severe) ( $p=0.08$ ). There was no significant difference of MLR in mild and severe DHF.

**Key words:** Dengue hemorrhagic fever, monocyte lymphocyte ratio, DHF degree

**INTRODUCTION**

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus, a virus that can be transmitted

through mosquito bites. DHF is one cause of illness and death in the world, including in Asia. Approximately 500,000 cases occur each year in the world. Dengue

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severity in Southeast Asia is the most in age 13–35 years.<sup>1</sup>

According to the WHO, there are four stages of infection DHF severity, degree I is characterized by fever accompanied by symptoms are not typical and positive Rumpel Leede; stage II the degree I in the presence of spontaneous bleeding in the skin<sup>10</sup> or other bleeding. Grade III is characterized by circulatory failure<sup>10</sup> a rapid and weak pulse and also a decrease in pulse pressure (<20 mmHg), hypotension (systolic decreased to <80 mmHg), cyanosis around the mouth, cold limbs, moist skin and the patient is agitated; while stage IV is characterized by severe shock (profound shock) that is no palpable pulse and blood pressure. Grade I and II are generally handled well, while stage III and IV are often an urgency that can lead to death.<sup>2–4</sup>

The pathogenesis of DHF infection allegedly involves monocytes and lymphocytes as a result of the immune response to infection.<sup>2,5</sup> Therefore, the number in peripheral blood would describe the state<sup>18</sup> of immune response to infection. The number of monocytes and lymphocytes in peripheral blood are described by Monocytes Lymphocyte Ratio (MLR). During this time MLR is proved beneficial description about of monocytes and lymphocytes immune responses. A previous research showed that MLR described immune response to malaria infection, prediction of tuberculosis and Human Immunodeficiency Virus (HIV) severity.<sup>6,7</sup> Differential counts and MLR are an examination routine and easy for detecting infectious diseases including DHF. This research is aimed to know whether there were differences between MLR in mild and severe DHF.

## METHODS

This study was a retrospective cross-sectional study. Subjects were DHF patients at the Dr. Kariadi Hospital

Semarang from January to December 2013. This study used consecutive nonsampling random with inclusion and exclusion criteria. The inclusion criteria were children 1–14 years old, in order with WHO criteria for DHF diagnosis. Patients with tuberculosis (TB), HIV, malaria and typhoid fever were excluded.

Monocyte Lymphocytes Ratio (MLR) was defined as the ratio between the number of monocytes divided by lymphocytes obtained from the examination of leukocyte count. Mild DHF was grade I and II of WHO criteria in group I, while severe DHF was grade III and IV of WHO criteria in group II. Patient's medical records were needed for history, physical examination and laboratory test results. Laboratory test results were examined by Beckman Coulter Hematology Analyzer HMX-1 (Beckman Coulter, Brea, CA) with venous blood samples. Monocyte lymphocytes ratio calculations was done manually by calculating the leukocyte count.

Data were analyzed by Student's t test to determine the MLR in mild and severe DHF. Significance was stated at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Eighty DHF patients were divided into two groups (mild and severe). Group I consisted of 40 patients with mild DHF. Group II consisted of 40 patients with severe DHF. Subject consisted of 41 males (51.2%) and 39 females (48.8%). Basic characteristic data are shown in Table 1.

Normality test result showed that the MLR data distribution was not normal, after transformation process, MLR data was normally distributed. Monocyte lymphocytes ratio value for mild DHF was 0.03 to 0.33 (median 0.11), while MLR value in severe DHF was 0.03 to 0.59 (median 0.16). Statistical analysis showed that there were no significance of MLR between group I and group II ( $p = 0.08$ ) (Table 2).

**Table 1.** Subject characteristics

Subject characteristics	%	Mean ± SD	Median (min-max)
Gender			
Male	39 (48.8)		
Female	41 (52.2)		
Age (year)			6.5 (1–14)
Hb (gr/dL)		12.38 (2.83)	
Ht (%)			38.2 (9.7–58.2)
Leucocyte count (103/ $\mu$ L)			5.1 (1.25–23.9)
Thrombocyte count (103/ $\mu$ L)			49,950 (4,300–165,000)
Lymphocytes (%)		41.46 (15.48)	
Monocytes (%)			5 (1–22)
MLR			0.126 (0.03–0.59)

**Table 2.** Statistical analysis of MLR in mild and severe DHF

Discription	Mild DHF Median (min- max)	Severe DHF Median (min- max)	p
MLR	0.11(0.03;0.33)	0.16(0.03;0.59)	0.08*

\* Student t test

Monocytes and lymphocytes are an immune system, that monocytes act as APC (antigen presenting cells) and produce cytokines in response to infection. Mature monocytes would change into macrophages in tissues. Mononuclear phagocytic cells are monocytes, macrophages, histiocytes and Kupffer cells which are the main site of dengue virus replication.<sup>8,9</sup> IL-1, IL-6 and TNF- $\alpha$  that were produced by monocytes can induce fever, otherwise, monocytes also play a role in remodeling and repairing tissue. The lymphocytes plays a role in immunologic processes and helps phagocytes cell.<sup>10,12</sup>

Monocyte lymphocytes ratio value was previously used for tuberculosis, HIV and malaria predictors. Marimwe *et al.*<sup>6</sup>, reported that MLR value described the immune response to malaria infection.<sup>6</sup> Naranbhai *et al.*<sup>7</sup>, reported that the MLR was connected as a prediction of disease severity tuberculosis and HIV infection.<sup>7</sup>

In this study, the value of MLR in mild and severe DHF infections showed no significant difference ( $p=0.08$ ). This was consistent with Jameel *et al.*<sup>11</sup> who reported that there was no change in the number of monocytes in DHF infection and the observation of DHF infection with and without shock there did not found differences in leukocyte counts and concentration.<sup>11</sup> Passos *et al.*<sup>12</sup>, also reported that lymphocyte counts between dengue and non dengue there was not found significantly different ( $p=0.08$ ).<sup>12</sup> The MLR value was not significant in this study due to the possibility of different infection pathophysiology of DHF.

In addition to the theory of dengue infection in monocytes and macrophages, there were several theories explaining the dengue virus infection, such as genetics theory that HLA-A1, HLA-B, HLA-CW1 and HLA-A29 were more exposed to DHF. Other studies mentioned that the loci HLA B35, AW-33, CW4 and DR7 was found in many cases of shock, while BW 51, BW 62 and DRA was common in cases of DHF without shock.<sup>2</sup> Complement theory showed that antigen-antibody complex would activate complement, activation of the complement thus increasing the production of C3a and C5a which increases capillary permeability, resulting in leakage of plasma, hypovolemia, hemoconcentration and shock. Another theory was the theory of secondary infections showing that the second DHF infection with other types of viruses would cause more severe clinical

manifestations. In addition, there was a virulence theory of the virus, the infection of the more virulent serotype virus could cause more severe DHF compared to avirulent virus. DEN-3 serotype was more the cause of death than the others serotypes.<sup>2</sup>

Limitation of this study was a cross sectional study design that could not clearly describe when the change of leukocyte counts occurred. In addition, in this study the subjects of unknown status of primary or secondary infection were not investigated.

## CONCLUSION AND SUGGESTION

The conclusion of this study was that no difference of MLR in mild and severe DHF infection was found. The suggestion is that further studies with larger result and considering the type of infection, whether it is primary or secondary dengue infection are needed.

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