THE KINETIC OF ACTIVATED MONOCYTES IN ACUTE PHASE DENGUE INFECTION

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ABSTRACT

Introductions: Nowadays dengue infection is at present one of the most common mosquito-borne viral diseases of humans worldwide. Initially, Dengue infections were primarily recorded when they occurred as epidemics in tropical and subtropical countries. Monocyte/macrophage infection is central to the pathogenesis of dengue fever and to the origin of dengue hemorrhagic fever. Increased activation of monocytes and greater numbers of DEN-infected cells were associated with more severe Dengue, implicating a role for monocyte activation in dengue immunopathogenesis. Thus, more knowledge about the relation between numbers of activated monocyte with dengue severities is essential for better understanding regarding this subject.

Objectives: Studying the relation between numbers of activated monocyte, marked by HLA-DR marker intensity with Dengue Fever severity throughout the disease course, from day 2 until day 6 of acute dengue fever.

Methods: This research is cross sectional observational study. The location in done at Dr. Sardjito Hospital in 6 months time.

Results: There is a negative correlation between median number of activated monocyte with dengue severity, on day 3 of acute dengue fever.

Conclusion: There was a negative correlation between numbers of activated monocyte marked by HLA-DR intensity with disease severity on day 3 of acute dengue infection.

Keywords: Activated Monocyte; Human Leukocyte Antigen (HLA)-DR intensity; Percentage of Activated Monocyte; Dengue Fever Severity.

INTISARI

Pendahuluan: Infeksi Dengue saat ini merupakan penyakit virus yang dibawa nyamuk yang tersering pada manusia di seluruh dunia. Monosit berperan penting pada patogenesis infeksi Dengue dan terjadinya DHF. Jumlah monosit terinfeksi Dengue berhubungan dengan derajat keparahan Dengue yang lebih berat, yang mengindikasikan adanya peranan monosit teraktivasi pada imunopatogenesis Dengue. Untuk membuktikan hubungan monosit teraktivasi dengan derajat infeksi Dengue diperlukan studi kinetik aktivasi monosit selama fase akut infeksi Dengue.

Tujuan: Meneliti hubungan antara jumlah monosit teraktivasi yang ditandai dari intensitas penanda HLA-DR dengan derajat demam Dengue selama fase akut infeksi Dengue.

Metode: Penelitian observasional potong lintang ini dilakukan di RSUP dr. Sardjito selama 6 bulan dengan subyek 32 orang, terdiri dari 17 orang Dengue Fever (53,1%) dan Dengue Hemorrhagic Fever (DHF) 15 orang (46,9%). Sampel darah tepi pasien diambil pada hari kedua demam sampai hari keenam. Monosit teraktivasi diukur secara flowcytometrik dengan mengukur intensitas ekspresi HLA-DR pada membran monosit. Hubungan antara monosit teraktivasi dengan keparahan infeksi Dengue dianalisis dengan korelasi Spearman dan *independent t-test*.

Hasil: Tidak terdapat perbedaan persentase monosit teraktivasi pada hari kedua sampai hari keenam. Terdapat korelasi negatif (r=-0,35; p=0,05) antara median jumlah monosit teraktivasi dengan derajat keparahan Dengue hanya pada hari ketiga demam Dengue akut. Rerata intensitas HLA-DR pada monosit lebih tinggi secara bermakna pada infeksi Dengue ringan dibandingkan DHF pada hari kelima.
Simpulan: Monosit teraktivasi pada fase akut infeksi Dengue dan mungkin berperan pada infeksi Dengue

Kata kunci: infeksi Dengue derajat keparahan Dengue monosit teraktivasi, intensitas *Human Leukocyte Antigen (HLA)-DR*

INTRODUCTION

Dengue infections are at present one of the most common mosquito-borne viral diseases of humans worldwide. Initially, dengue infections were primarily recorded when they occurred as epidemics in tropical and subtropical countries. But over time, increasing globalization and human movement coupled by the increase in the geographic area where the *Aedes aegypti* mosquito vector inhabit, has promoted dengue virus (DEN) infection to nearly every corner of the world. Approximately 50 to 100 million people contract dengue fever annually, and about 200,000 to 500,000 contract dengue hemorrhagic fever (DHF), and the mortality rate is about 5%, predominantly in children under 15 years of age [1].

Monocyte/macrophage infection is central to the pathogenesis of dengue fever and to the origin of DHF/DSS (Dengue Shock Syndrome). Previous infection with a heterologous dengue-virus serotype may result in the production of nonprotective antiviral antibodies that nevertheless bind to the virion's surface and through interaction with the Fc receptor focus secondary dengue viruses on the target cells [2].

Recent studies showed that using monoclonal antibodies to structural and nonstructural DEN proteins, activated monocytes (CD86+, CD32+, CD14+, CD11c+) were the main target for DEN infection regardless of immune status. There is also significant increase in monocytes activation (CD86+) and a higher prevalence of CD32+, CD14+, CD11c+, and DEN prM+ cells in DHF patients compared with DF patients. Thus, increased activation of monocytes and greater numbers of DEN-infected cells were associated with more severe dengue, implicating a role for monocytes activation in dengue immunopathogenesis [3].

MATERIALS AND METHODS

This research uses the cross sectional observational study. The location took place at Sardjito Hospital in 6 months time. The subject being used was the patient admitted to Sardjito Hospital and suspected of having dengue infection according to the WHO criteria. This research was conducted from May 2010 until October 2010 at Dr. Sardjito Hospital, Yogyakarta.

The inclusion criteria were the subjects aged more than 14 years old and suspected to have dengue infection with NS-1 positive (+ve). The exclusion criterion was the subjects who are also suffering from other diseases, other than dengue infection.

Blood sample was taken from patient admitted to Sardjito Hospital and suspected of having dengue infection with NS-1 positive. Blood was taken aseptically by vein puncture and put into K3 EDTA Vacutainer blood collection tube.

The blood sample was kept in a room 20° – 25° C. The sample was sent to the Pathology Clinic Laboratory for complete blood test using the hematology analyzer. After that, analysis of HLA-DR activation marker was done by flow cytometric. The reagent used to detect activated monocyte was CD8 FITC/ HLA-DR /45 per CP antibody. The flowcytometric readings were recorded using the Cell Quest method.

We calculated the demographic features of the participant on the classification of the dengue infection. Next, result obtained was recorded in terms of median number and percentage of activated monocyte. Here the descriptive analysis was done where the kinetics of activated monocyte was observed. Spearman's Correlation Test was conducted to see the correlation between number of activated monocyte, marked by HLA-DR intensity and percentage of activated monocyte with dengue severity for each day throughout the hospital admission (day 2 until day 6). Independent t-test was also done to observe any different between numbers of activated monocyte with dengue severity from day 2 until day 6.

RESULTS AND DISCUSSIONS

Table 1 shows that 53.1% of the subjects tested were classified under dengue fever and 46.1% under dengue hemorrhagic fever, which consist of DHF patient grade 1 and grade 2.

Table 1: Classification of Dengue Infection in subject

Type of Dengue	Frequency (n)	Percentage (%)
DF	17	53.1
DHF	15	46.9
Total	32	100

Table 2: Median number of activated monocyte for each day of acute dengue infection.

Days	Mean	Std. Deviation
2	334.9381	137.51644
3	304.8009	130.49518
4	314.4097	132.27171
5	290.0728	111.15938
6	262.9119	87.65818

Table 3: Percentage numbers of activated monocyte for each day of acute dengue infection.

Days	Mean(%)	Std. Deviation(%)
2	92.69	7.71
3	90.15	9.46
4	93.17	5.27
5	92.43	7.34
6	90.06	8.68

Table 4: Spearman's Correlation Test on median number of activated monocyte compared with dengue severity for each day of acute dengue infection.

Days	correlation Spearman's rho	p-value
2	-0.312	0.082
3	-0.349	0.050*
4	+0.105	0.567
5	-0.319	0.075
6	+0.051	0.782

(* = Significant value)

Table 2 shows the median number of activated monocyte for each day of acute dengue infection. We can observe the trend of decreasing mean number of activated monocyte from day 2 until day 6.

Table 3 shows the percentage numbers of activated monocyte. Overall, there is a trend of decreasing number of activated monocyte from day 2 until day 6.

Table 4 shows the summary of Spearman's Correlation Test on median number of activated monocyte compared with dengue severity for each day of acute dengue infection. From above, we can observe only median day 3 showed statistical significant result with a negative correlation between dengue severity and number of activated monocyte.

Table 5 : Median number of activated monocytes.

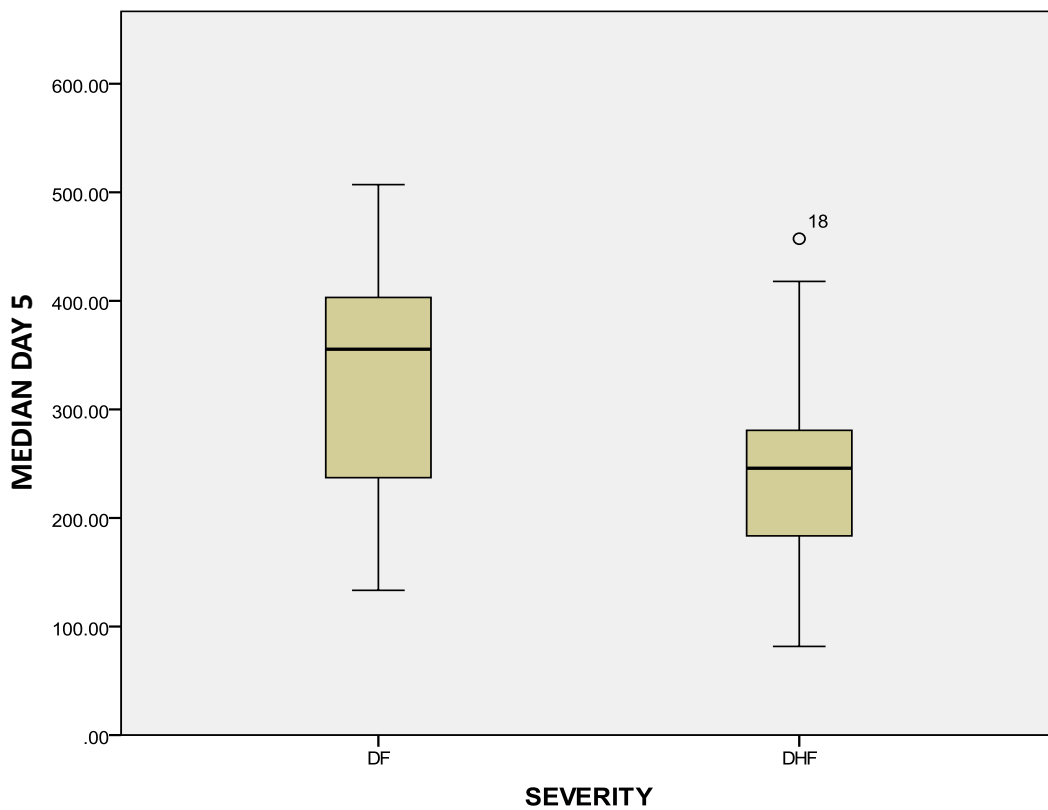| Day	severity	mean	Std. deviation	*p*-value
2	DF	376.52	35.89	0.40
	DHF	287.81	28.71	
3	DF	343.16	26.11	0.07
	DHF	261.34	37.10	
4	DF	305.44	35.50	0.70
	DHF	324.57	30.60	
5	DF	327.35	27.13	0.04*
	DHF	247.82	25.11	
6	DF	258.25	14.44	0.76
	DHF	268.19	29.34	

(* = Significant value)

Figure 1: The difference between DF and DHF median values on day 5.

Table 5 displays the result of Independent t-test done on the median number of activated monocyte. There is a significant value day 5, suggesting that there is a difference in number of activated monocyte between DF and DHF patients today.

Figure 1 will show the distribution numbers of activated monocyte and the median value for DF and DHF patients.

There is a total of 32 participants in this study but some of the data is not present for day 5 and day 6. Participants for day 5 and day 6 is originally 30 and 27 participants (should be a total of 32). Statistical analysis would not be valid if the participant data is not present so the mean of day 5 and day 6 is inserted into the data value.

Table 4 shows the summary of Spearman's Correlation Test on median number of activated monocyte compared with dengue severity for each day of acute dengue infection. Day 3 of acute infection showed a significant result ($p \leq 0.05$) on negative correlation between numbers of activated monocyte with dengue severity. This means that as the number of activated monocyte decrease, more severe dengue infection will manifest as it is inversely proportional. This finding is in line with other previous study, which stated that low level of HLA-DR expression on monocyte will result in poorer outcome of sepsis patient and infectious complication in ruptured abdominal aortic aneurysms patients[4]. But, we do not obtain a similar result to previous study which mentioned that number of activated monocyte and greater number of DEN-infected cells associate with more severe dengue was because the method that was used were different. For this study, we used HLA-DR as marker and we checked for CD45, whereas the previous study chose to measure CD86+ for their activated monocyte marker and CD32 for the cytokines[3].

Table 5 displays the result of Independent t-test done on the median number of activated monocyte. On day 5, there is a significant value ($p<0.05$) when DF patients was compared to DHF patients. Thus, there was a significant difference in the median numbers of activated monocyte with for DF patients (M=327.35, SD=27.13) and DHF patients (M=247.82, SD=25.11); t(30)=2.13, p=0.041. This means that there is a significant difference in number of activated monocyte on day 5 when compared between DF and DHF patients. Specifically, DF patients have higher median number of activated monocyte on this day. This is due to the fact that during severe dengue fever, monocyte deactivation may occur by HLA-DR down regulation and by Tissue Factor expression and consumption of coagulation factors[5].

Based on the result that have been stated above, number of activated monocyte showed a negative correlation when compared to dengue severity in acute dengue infection. There are also a number of other risk factors that may contribute and help in determining the outcome of dengue infection, namely are like virus virulence as stated by Kurane (2006)[6] and Noisakran (2007)[1]. Kurane (2006)6 stated that Southeast Asia DEN strain type 2 may contribute significantly toward disease progressiveness to DHF in different countries of Americas.

Other than that, infection by different secondary DEN strain may also exacerbate dengue severity [1]. In this study, it was mentioned that secondary infection by DEN, especially by DEN of different serotype may lead to more serious outcome of dengue fever. Lei (2001)[7] also mentioned that secondary infection by DEN type 2 also possess more risk in developing DHF/DSS in patients, supported by the fact that patients with a secondary antibody response were twice as likely to have DHF, compared with those with a primary antibody response.

CONCLUSION

There was a significant negative correlation between numbers of activated monocytes, marked by median value of HLA-DR intensity on day 3 of dengue infection.

There was a statistical difference between median number of activated monocyte for Dengue Fever and Dengue Hemorrhagic Fever on day 5.

There was no statistical correlation between percentage numbers of activated monocyte when compared with dengue severity.

SUGGESTION

The outcome of the study would be more favorable if there are more patients as subjects of study and higher grade of Dengue Hemorrhagic Fever (grade 3 and 4) to be included and enrolled in the study.

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