Interleukin-18 levels in adult dengue fever and dengue hemorrhagic fever

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Abstrak

Interleukin (IL)-18 (interferon-γ inducing factor) merupakan salah satu sitokin yang diproduksi makrofag, berperan dalam diferensiasi sel T-helper menjadi T-helper-1, dan produksi interferon γ. T-helper-1 berperan dalam imunitas seluler khususnya pada infeksi virus termasuk infeksi dengue. Dilakukan studi deskriptif korelatif mengenai hubungan kadar IL-18 dengan derajat penyakit pada penderita demam dengue (DD) dan demam berdarah dengue (DBD) yang dirawat. Pada 42 subyek yang terdiri dari 20 (47,6%) penderita demam dengue dan 22 (52,3%) demam berdarah dengue (derajat I sampai IV menurut kriteria WHO tahun 1999). Didapatkan kadar IL-18 secara bermakna lebih tinggi pada DHF dibandingkan DF. Didapatkan korelasi kadar IL-18 dengan nilai hematokrit dan hitung trombosit. Studi ini menunjang kemungkinan keterlibatan IL-18 dalam patogenesis DBD pada pasien dewasa. ***(Med J Indones 2004; 13: 86-9)***

Abstract

Interleukin (IL)-18 (interferon-γ inducing factor) is one of cytokines, produced by macrophage, take part in differentiation T-helper (Th) to Th1 and interferon γ producing. T-helper1 play role in cellular immunity especially in viral infection include dengue. A descriptive correlative study has done to know the correlation between IL-18 levels and disease severity in admitted dengue fever (DF) and dengue hemorrhagic fever (DHF) patients. In 42 subjects consist of 20 (47.6%) DF and 22 (53.3%) DHF (grade I to IV WHO criteria, 1999) showed that IL-18 levels significantly higher in DHF than DF patients. There are significant correlation between IL-18 levels and hematocrit and low platelet value. This study supports the possible role of IL-18 in pathogenesis DHF in adults. ***(Med J Indones 2004; 13: 86-9)***

Keywords: *dengue; dengue hemorrhagic fever; IL-18; cytokine; pathogenesis*

Dengue virus infection can manifest as wide spectrum from asymptomatic, mild self-limiting febrile illness, dengue fever (DF) or severe dengue hemorrhagic fever (DHF).[1] The characteristic features of DHF are increasing of capillary permeability caused hemococentration, increased hematocrit and thrombocytopenia. Extensive plasma leakage into serous cavities of the body also caused hypotension, profound shock and death.

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The pathogenesis of DHF is still not fully understood. Several clinical studies support the hypothesis that cytokine production is important in the pathogenesis of DHF.[2] Interleukin (IL)-18 originally described as IFN γ inducing factor, a pro-inflammatory cytokine and an important stimulator or mediator of Th1 and Th2 immune response, enhances NK cell cytotoxicity.[3-4]

The main source of IL-18 is macrophage like cells. In the present study have investigated IL-18 protein expression in primary human macrophages in response to influenza A and Sendai virus infection.[5] Macrophages expressed pro IL-18 but produce biologically active IL-18 only after virus infection. The IL-18 release was due to virus infection induces proteolytic processing of 24 kDa pro IL-18 into its mature 18 kDa form. Pro IL-18 processing required active caspase-1 enzyme.[6] IL-18 collaboration with IL-12 to produce of IFN γ from $CD4^+$ Th1 cells, B cells and NK cells.[7]

Unlike IL-12 which was high in DF and absent in patients with DHF especially grade III and IV; all of

the Th_2 type cytokines are high in DHF/severe dengue infection.[8-10] A recent study has shown that IL-18 is a potent co inducer of IL-13 in NK cells and Th cells (Th_2). It can act as a strong co inducer of Th_1 or Th_2 cytokines.[11-12] This could explain its role in dengue virus infection.

In this study we will investigate IL-18 levels in adult dengue fever and dengue hemorrhagic fever, and the correlation with severity and degree of hemo-concentration and thrombocytopenia.

METHODS

Study subject were enrolled from acute dengue cases, admitted in Dr. Cipto Mangunkusumo and Persahabatan general hospital during December 1999 until July 2000. Patients clinically presented as dengue fever or dengue hemorrhagic fever were enrolled to this study. Clinical data were recorded include age, sex, duration of fever, other symptoms and bleeding manifestations. The laboratory test were performed include serial hematocrit, platelets counts and dengue serology. The serum specimen were collected once daily, storage and freeze in -40^{o}C for IL-18 cytokine examination.

Depending on clinical manifestation, serial hematocrit levels, platelet counts, the presence of ascites or pleural effusion, the subjects were diagnosed as dengue fever, dengue hemorrhagic fever grades I, II, III and IV. Dengue fever determined if there is no evidence of plasma leakage. DHF if there are evidence of plasma leakage include the increasing of serial hematocrit levels more than 20% between the acute and convalescence phase, ascites or pleural effusion. DHF grade I, II, III and IV determined by the criteria of WHO.

Evidence of dengue virus infection determined by dengue rapid Immunochromatographic test or IgM capture and IgG capture ELISA test, using commercial kits (Pan-Bio, Australia). Dengue infection were classified as primary if the IgM was positive, secondary if the IgM and IgG were positive.

Determination of IL-18 cytokine levels were performed in Department of Bacteriology, Iwate Medical University, Morioka, Japan. The freeze sera were transported using dry ice and stored at -80^{0}C until they were tested. Commercial Elisa Kit (MBL medical & Biological Laboratories co, ltd) were used to measure IL-18 levels in the sera of patients. The cut off point value is > 249,5 pgml^{-1}. The data were analyzed using computer statistics program. Comparison IL-18 levels between groups were analyzed by Mann-Whitney test. The correlation between IL-18 levels and hematocrit or platelet counts were analyzed by Spearman's correlation test. Statistical significance was defined as $p<0.05$.

RESULTS

Forty-two subjects were enrolled to this study during the period (17 male and 25 female, median age 21 years old) (Table 1). Duration of fever from the first day of fever to the day of admission between 2 to 6 days. Twenty cases (47.62 %) were defined as dengue fever and 22 cases (52.38 %) as dengue hemorrhagic fever. Most of DHF cases was grade I and II (18 of 22 cases) and dengue shock found in 3 subjects. Most of bleeding manifestation were positive tourniquet test and petechia; other istes include nose, vaginal, and GI tract. (Table 1).

Table 1. Subjects Characteristics

	Total	Percent
Age (year)		
14 – 19	14	33.33
20 – 25	17	40.48
26 – 30	9	21.43
≥ 31	2	4.76
Sex		
Male	17	40.48
Female	25	59.52
Duration of fever (day)		
2	4	9.52
3	13	30.95
4	15	35.71
5	6	14.29
6	4	9.52
Clinical symptom and sign		
Nausea	40	95.24
Vomiting	26	61.90
Epigastric pain	16	38.09
Shock	3	7.14
Hepatomegaly	3	7.14
Bleeding manifestation		
Tourniquet test positive	16	38.10
Petechiae	23	54.76
Gum bleeding	2	4.76
Epistaxis	7	16.67
Hematemesis	1	2.38
Melena	1	2.38
Menometrorhagia	3	7.14
Grade		
DF	20	47.62
DHF	22	52.38
Grade I	6	14.29
Gare II	12	28.57
Grade III	3	7.14
Grade IV	1	2.38

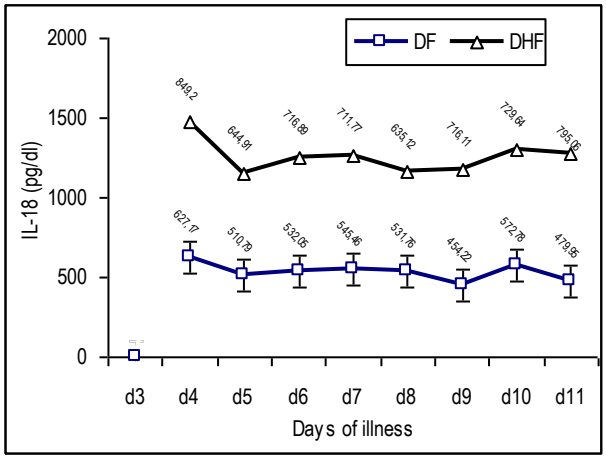

Figure 1. Mean levels of IL-18 according to grades of illness (DF and DHF)

Figure 1 show the IL-18 levels according to the day of the illness in DHF and DF subjects. Interleukin-18 levels were highest during the first 4 days of illness with the mean value 849.20 $\pm$ 353.27 $pgml^{-1}$ in DHF; and 627.18 $\pm$ 282.35 $pgml^{-1}$ in DF (Mann-Withney test $p>0.05$). The lower levels of IL-18 were found on day 9^{th}, which the mean levels in DHF was 716.10 $\pm$ 282.48$pgml^{-1}$ and 454.22$\pm$210.52 $pgml^{-1}$ in DF (Mann-Withney test $p<0.05$). The levels were increased again on 8^{th} day of illness. Comparison between IL-18 levels during all the observation days in DF and DHF showed the significant differences (Mann-Withney test p=0.000) (Figure 2). Comparison between DHF grade I-II and III-IV showed insignificant differences (Mann-Withney test>0.05).

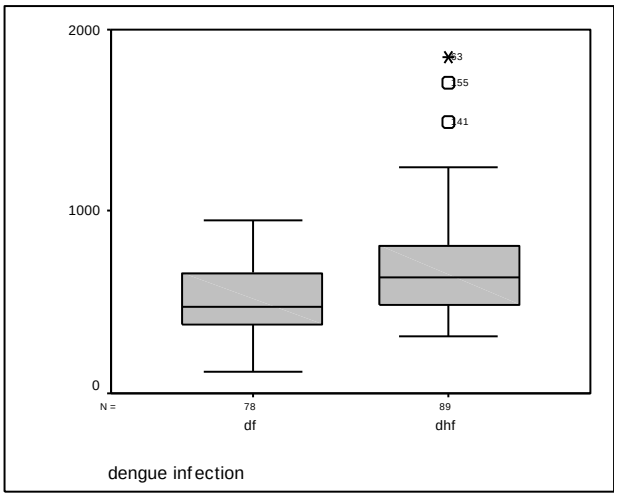

Figure 2. Boxplot showing IL-18 levels in DF and DHF. (Mann-Withney test p=0.000)

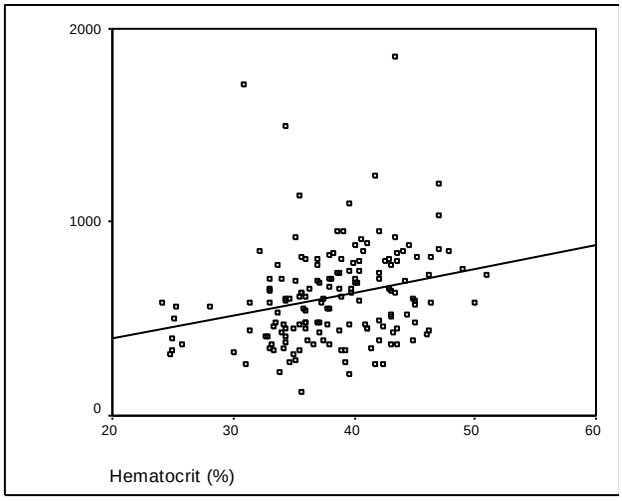

Figure 3 . Scatter diagram show correlation fit line between between IL-18 levels and hematocrit. Spearman correlation test show significant moderate correlation with r_s= 0.313 p=0.000.

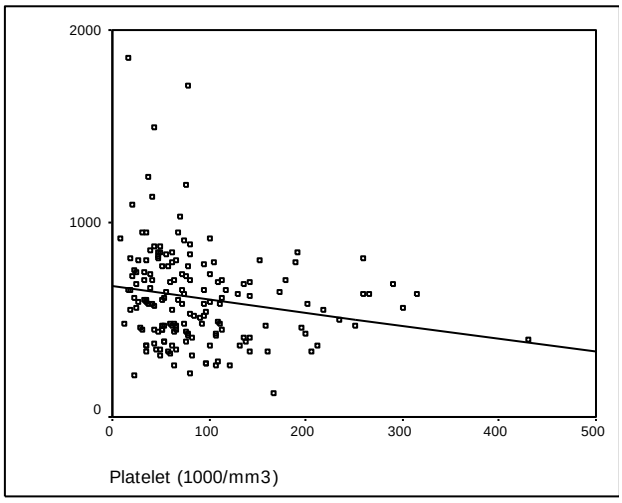

Figure 4 . Scatter diagram show correlation fit line between IL-18 levels and platelet count. Spearman correlation test show significant moderate correlation with r_s=-0.222 p=0.004.

Correlation between IL-18 levels, hematocrit and platelet count show significant correlation with r_s= 0.313 and p=0.000 (Figure 3); and r_s=-0.222 p=0.004 (Figure 4). Correlation between IL-18 levels, hematocrit and platelet count according to day of illness show in table 2. There are moderate to strong correlation between the IL-18 levels, hematocrit and count.

Table 2. Correlation between levels of IL-18 with platelet count and hematocrit

Days of IL-18	Platelet	Correlation	Hematocrit	Correlation
1	r = - 0,15	Weak	r = + 0,40	Moderate
2	r = - 0, 52	Strong	r = + 0,58	Strong
3	r = - 0,25	Moderate	r = + 0,29	Moderate
4	r = - 0,44	Moderate	r = + 0,30	Moderate
5	r = - 0,31	Moderate	r = + 0,09	Weak
6	r = - 0,19	Weak	r = - 0,63	-
7	r = - 0,29	Moderate	r = - 0,88	-

DISCUSSION

Interleukin-18 is primary produced by monocyte / macrophages in response to dengue infection.[5] The results of this study demonstrate elevated levels of IL-18 in the patients with DF and DHF and correlated with hematocrit and platelet value. The levels of IL-18 were significantly higher in patients with DHF compare to DF. These findings suggest that IL-18 is produced in response to dengue virus infection and may have a important role against severe dengue disease.

Interleukin-18 is an important immune-regulatory cytokine and has been shown to enhance host cellular responses, including the ability to promote clearance of virus.[4] The studies on the dengue patients showed the shift from the predominant Th_1 type cytokine response observed in DF to Th_2 type response in patients with severe DHF.[2,10] Based on these results, it has been proposed that dengue virus induces the production of a cytokine cascade that shifts from Th_1 dominant response to Th_2 response, resulting in severe DHF. The results of this study are consistent with the hypothesis that the presence of IL-18 is associated with Th_1 type response and Th_2 type response (levels of IL-18 in patients dengue correlated with the severity of the disease). These findings support the possible role of IL-18 in pathogenesis of dengue hemorrhagic fever.

CONCLUSIONS

Interleukin-18 levels increased both in DF and DHF patients, especially in the first 4 day of illness. Interleukin-18 levels significantly higer in DHF compare to DF patients and correlated with hematocit and platelet value.

REFERENCES

1. Nimmannitya S. Clinical manifestation of dengue/dengue haemorrhagic fever. In:Thangcharoen P, editor. Monograph on dengue/dengue haemorrhagic fever. New Delhi: World Health Organization Regional Office for South East Asia; 1993.p.48-54.
2. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustofa AS. Cytokine cascade in dengue hemorrhagic fever: Implication for pathogenesis.FEMS Immunol Med Microbiol 2000; 28:183-8.
3. Hoshino T, Wiltrout RH, Young HA.IL-18 is a potent co inducer of IL-13 in NK & T cells: a new potential role for IL-18 in modulating the immune response. J Immunol 1999 : 5070-7.
4. Garcia VE, Uyemura K, Sieling PA, Ochoa MT, Morita HO, Kurimoto M et al. IL-18 promotes type I cytokine production from NK cell and T cell in human intra cellulair infection. J Immunol 1999 ; 62 : 6114-21.
5. Gillespie MT. Horwood NJ. Interleukin 18: Perspectives on the newest interleukin. Cytokine and Growth Fac Rev 1998;9:109-16.
6. Pirhonen J, Sareneva T, Kurimoto M, Julkunen I, Matikainen S. Virus infection induces proteolytic processing of IL-18 in human macrophages via caspase-1 and caspase-3 activation. Eur J Immunol 2001; 31: 726-33.
7. Tominoga K, Yoshimoto T, Torigae K, Kurimoto M, Matsui K, Hada T et al. IL-12 synergizes with IL-18 or IL-13 for IFN-γ production from human T cells. Inter Immunol 2000; 12: 151-60.
8. Pasca AS, Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R, Mustafa AS. Role of interleukin-12 in patients with dengue hemorrhagic fever. FEMS immunol Med. Microbiol. 2000; 20: 151-5
9. Mustafa AS, Elbishbishi EA, Agarwal R, Chaturvedi UC. Elevated level of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. FEMS immunol Med Microbiol 2001; 30: 229-33.
10. Suvatte V. Immunological aspects of dengue hemorrhagic fever: Studies in Thailand. Southeast Asian. J Trop Med Pub Hlth 1987; 18 : 312-4
11. Kurane I, Innis BL, Nimmannitya S, Nisalak A, Meager A, Janus J et al. Activation of T lymphocytes in dengue virus infections. J Clin Invest 1991 ; 88 : 1473-80.
12. Nakanishi K, Yoshimoto T, Titsui H, Okamura H: Interleukin-18 regulates both Th 1 and Th 2 responses. Annu Rev Immunol (abstracts) 2001; 19: 423-74