The Differences Between Interleukin-6 and C-reactive Protein Levels Among Adult Patients of Dengue Infection with and without Plasma Leakage

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ABSTRAK

Tujuan: *mengetahui perbedaan kadar IL-6 dan protein C reaktif antara kelompok infeksi dengue dengan dan tanpa kebocoran plasma.* ***Metode:*** *penelitian potong lintang terhadap penderita infeksi dengue yang dirawat di RS Cipto Mangunkusomo dan RS Persahabatan antara 1 Maret 2014 - 1 April 2015 dengan menganalisis perbedaan kadar IL-6 dan protein C reaktif pada hari ke-3 dan ke-5 demam antara kedua kelompok, maupun pada tiap kelompok. Kadar IL-6 dan protein C reaktif pada kedua kelompok dianalisis menggunakan uji t tidak berpasangan atau Mann-Whitney, dan pada masing-masing kelompok dianalisis dengan uji t berpasangan atau Wilcoxon.* ***Hasil:*** *sampel yang diteliti terdiri atas 24 orang dengan kebocoran plasma dan 20 orang tanpa kebocoran plasma. Kadar IL-6 pada kelompok dengan dan tanpa kebocoran plasma pada hari ke-3 dan ke-5 demam adalah 8,56 (1,85-96,15) vs. 3,80 (1,94-81,93) pg/mL (p=0,069) dan 4,30 (1,60-70,28) vs. 2,76 (1,26-11,67) pg/mL (p=0,025), sedangkan untuk protein C reaktif adalah 10,1 (4,3-36,5) vs. 6,8 (3,0-21,6) mg/L (p=0,014) dan 5,0 (2,0-20,1) vs. 2,9 (0,1-9,9) mg/L (p=0,048). Kadar IL-6 hari ke-3 dan ke-5 demam pada kelompok dengan dan tanpa kebocoran plasma adalah 8,56 (1,85-96,15) vs. 4,30 (1,60-70,28) pg/mL (p=0,037) dan 3,80 (1,94-81,93) vs. 2,76 (1,26-11,67) pg/mL (p=0,005). Kadar protein C reaktif hari ke-3 dan ke-5 demam pada kelompok dengan dan tanpa kebocoran plasma adalah 10,1 (4,3-36,5) vs. 5,0 (2,0-20,1) mg/L (p=0,0001) dan 6,8 (0,3-21,6) vs. 2,9 (0,1-9,9) mg/L (p=0,0001).* ***Kesimpulan:*** *pada hari ke-3 demam kadar IL-6 tidak berbeda antara kedua kelompok, sedangkan pada hari ke-5 demam, kadar IL-6 lebih tinggi pada kelompok dengan kebocoran plasma. Kadar protein C reaktif hari ke-3 dan ke-5 demam lebih tinggi pada kelompok dengan kebocoran plasma. Kadar IL-6 dan protein C reaktif kedua kelompok lebih tinggi pada hari ke-3 dibandingkan hari ke-5 demam.*

Kata kunci: *interleukin-6, protein C reaktif, dengue, kebocoran plasma.*

ABSTRACT

Aim: *to determine the differences in IL-6 and CRP levels among groups of dengue infection patients with and without plasma leakage.* ***Methods:*** *a cross-sectional study was conducted in adult patients with dengue infection who were treated at Cipto Mangunkusumo and Persahabatan Hospital between 1 March 2014 and 1 April 2015. The study analyzed differences in IL-6 and CRP levels on the 3rd and 5th day of fever in both groups, as well as differences in each group. Interleukin-6 and CRP levels in both groups, was analyzed using unpaired t-test or Mann-Whitney and in each group, the data was subsequently analyzed using paired t-test or Wilcoxon test.* ***Results:*** *the*

samples of study consisted of 24 subjects with plasma leakage and 20 subjects without plasma leakage. The level of IL-6 for groups with and without plasma leakage for the 3rd and the 5th day of fever were 8.56 (1.85-96.15) vs. 3.80 (1.94-81.93) pg/mL (p=0.069) and 4.30 (1.60-70.28) vs. 2.76 (1.26-11.67) pg/mL (p=0.025), respectively; while for CRP level, there were 10.1 (4.3-36.5) vs 6.8 (3.0-21.6) mg/L (p=0.014) and 5.0 (2.0-20.1) vs 2.9 (0.1-9.9) mg/L (p=0.048). The level of IL-6 on the 3rd and the 5th day of fever in the group with and without plasma leakage were 8.56 (1.85-96.15) vs. 4.30 (1.60-70.28) pg/mL (p=0.037) and 3.80 (1.94-81.93) vs. 2.76 (1.26-11.67) pg/mL (p=0.005). The level of CRP on the 3rd and 5th day of fever in the group with and without plasma leakage were 10.1 (4.3-36.5) vs. 5.0 (2.0-20.1) mg/L (p=0.0001) and 6.8 (0.3-21.6) vs. 2.9 (0.1-9.9) mg/L (p=0.0001). ***Conclusion:*** *there was no difference in IL-6 level on the 3rd day of fever between the two groups; while on the 5th day of fever, the IL-6 level was higher in the group with plasma leakage. The level of CRP on the 3rd and the 5th day of fever were higher in the group with plasma leakage. The levels of IL-6 and CRP on the 3rd day of fever were higher than the levels on the 5th day of fever in both groups.*

Keywords: *interleukin-6, C-reactive protein, dengue, plasma leakage.*

INTRODUCTION

Until now, dengue infection still is an important health problem, either worldwide[1] or in Indonesia.[2] Dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most common manifestations of dengue infection. DF is a mild feature of the disease without plasma leakage; while DHF is a form of severe disease, accompanied by plasma leakage in critical phase.[3] Plasma leakage in DHF may include increased hematocrit, hypoalbuminemia as well as serous effusion in the pleural, pericardial and peritoneal cavities.[4,5] Poor treatment will cause dengue shock syndrome (DSS) and its mortality rate worldwide is still relatively high, i.e. 5-30%.[6] According to the immunopathogenesis theory, plasma leakage in DHF/DSS is a result of cytokine storm reaction or multiplication of cytokine production in the vascular endothelium.[7,8] The theory is supported by some studies in children,[9-12] which reported an association between increased levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and interleukin (IL)-6 with plasma leakage in DHF/DSS patients. Those three pro-inflammatory cytokines also serve as endogenous pyrogen, which will have increased levels in each process of acute inflammation and can induce synthesis of various acute phase proteins such as C-reactive protein (CRP).[13] The CRP itself is a systemic inflammatory marker that is commonly used in daily clinical practice and has been acknowledged as one of many inflammatory variables.[13,14]

In adults, few studies have been conducted on the pathophysiology of cytokine storm reaction. For clinical application, IL-6 is the most relevant to be studied among those three pro-inflammatory cytokines as it is the main inducer of C-reactive protein synthesis.[13] Until now, no study has been conducted in adult patients to compare the levels of IL-6 and CRP between groups of dengue infection with and without plasma leakage in order to identify the correlation between acute inflammatory process and the development of plasma leakage. Moreover, the temporal pattern of IL-6 and CRP production associated with cytokine storm reaction in dengue infection has not been known either. Therefore, serial measurements of IL-6 and CRP levels in febrile and critical phases should be performed since the IL-6 and CRP can also increase due to secondary bacterial infection.

In daily practice, we still can find a relatively high incidence of antibiotic treatment for patients with dengue infection due to a great concern on secondary infection caused by bacterial translocation from the intestinal lumen into the circulation.[4,15,16] The peak of febrile phase is on the 3rd day of fever; while critical phase usually occurs on the 5th day of fever.[17] Theoretically, the cytokine storm reaction in dengue infection occurs during febrile phase and will resolve in critical phase.[7,8] The theory should be confirmed further through clinical studies in adult patients with dengue infection. The aim of our study was to determine differences in the levels of IL-6 and CRP among groups of dengue infection with and without plasma leakage.

METHODS

The present study was a cross-sectional study using secondary data from medical records and case report form (CRF) of adult patients with dengue infection who were hospitalized in Cipto Mangunkusumo and Persahabatan Hospital between 1 March 2014 and 1 April 2015. Secondary data collection was performed in May – June 2015. The inclusion criteria were patients with dengue infection who came to the hospital with ≤3-day fever, aged ≥14 years and did not receive antibiotic treatment during hospitalization; while the exclusion criteria were pregnancy, patients with comorbidities (hypertension, diabetes mellitus, cardiovascular disease, liver cirrhosis, malnutrition with hypoalbuminemia, pulmonary edema, ARDS, other infectious disease, inflammatory disease, malignancy, patients with leukocytosis and or neutrophilia), patients who were on corticosteroid and immunosuppressant treatment and incomplete data.

Samples were obtained in our study by consecutive sampling. Sample size was determined using estimation formula of sample size for different mean value of 2 unpaired groups. For IL-6 variable, the minimal sample size for each group was 22 subjects with a total sample size of 44 subjects; while for CRP variable, the minimal sample size for each group was 18 subjects and the total sample size was 36 subjects. Based on both calculations, we took the greatest minimal sample size in order to achieve the study goal, i.e. 44 subjects.

Our study used secondary data and therefore, informed consent was not necessary. Ethical approval was issued by the Ethics Committee for Medical Research, Faculty of Medicine, University of Indonesia with a reference number of 443/UN2.F1/ETIK/2015; however, all data used for the purpose of the study were kept confidential.

Data Collection

Secondary data collected from medical record and CRF were basic data (age, sex, diagnosis, length of hospitalization), data obtained from history taking (duration of fever, bleeding manifestation), data from physical examination (vital signs, bleeding manifestation, pleural effusion, ascites), data from laboratory workup (hemoglobin, hematocrit, leukocytes, platelet count, albumin, IL-6 and CRP levels), as well as data from USG examination (pulmonary and abdominal USG).

IL-6 examination was performed by ELISA method using Quantikine reagent (R&D Systems, Inc); while the examination of CRP was done by immunoturbidimetry method using Cobas Integra instrument and CRPHS Latex (Roche) reagent. The subjects were considered as having dengue infection when they had 2-7 days of fever and were NS1 positive. Plasma leakage was defined when there was pleural effusion or ascites confirmed by pulmonary and abdominal USG.

Statistical Analysis

The collected data was filled into the subject form. Next, the data was tabulated into dummy table, which was consistent with the aim of the study and subsequently the data was edited and coded. Afterwards, the data was documented into a computerized magnetic disc and was processed by a personal computer using SPSS 22.0 software. The subject characteristic data was presented descriptively. Data of IL-6 and CRP levels in both groups were analyzed with Shapiro-Wilk test to evaluate their distribution and unpaired student t-test or Mann-Whitney test were used to compare between groups. Data of IL-6 and CRP levels on the 3rd and 5th day of fever in each group were analyzed using paired student t-test or Wilcoxon test. Differences between both groups were considered significant when the $p<0.05$.

RESULTS

The number of subjects participated in the study were 44 subjects. Subject characteristics are presented in **Table 1**. Mean age of the subjects in both groups could be categorized into young adults with a proportion of male and female as many as 50% respectively. On the 3rd and 5th day of fever, we found that the mean hemoglobin and hematocrit levels for both groups were still within normal limit; while the mean leukocyte and platelet counts were lower than normal values. On the 3rd day of fever, we found that the

Table 1. Subject characteristics

Characteristics	With plasma leakage (n=24)	Without plasma leakage (n=20)
Age (year), mean (SD)	23.7 (8.4)	24.3 (6.5)
Sex (male), n (%)	13 (54.2)	8 (40)
Hemoglobin level (g/dL), mean (SD)		
- The 3rd day	14.3 (2.0)	13.4 (1.3)
- The 4th day	13.9 (2.0)	13.3 (1.6)
Hematocrit (%), mean (SD)		
- The 3rd day	40.5 (4.9)	39.1 (3.0)
- The 5th day	39.8 (5.6)	9.5 (4.1)
Leukocytes (10^3/μL), mean (SD)		
- The 3rd day	3.37 (1.61)	3.02 (1.63)
- The 5th day	4.53 (2.08)	3.56 (1.51)
Platelets (10^3/μL), mean (SD)		
- The 3rd day	92.83 (30.89)	109.30 (31.47)
- The 5th day	4.88 (22.34)	60.00 (22.88)
Albumin level (g/dL), mean (SD)		
- The 3rd day	3.81 (0.26)	3.78 (0.25)
- The 5th day	3.30 (0.39)	3.50 (0.30)
Delta Hematokrit (%), mean (SD)	10.96 (6.59)	7.09 (4.37)
Delta Albumin (g/dL), mean (SD)	0.55 (0.33)	0.31 (0.26)

mean albumin levels for both groups were also still within normal limits, but on the 5th day of fever, the value was below normal limits for the group with plasma leakage; while for the group without plasma leakage, the value was still within normal limit. Delta of hematocrit and albumin levels was calculated based on differences of values on the 3rd and 5th day of fever. The mean delta of hematocrit levels in both groups was found less than 10%; while the mean delta of albumin levels for the group with plasma leakage was more than 0.5 g/dL and for the group without plasma leakage was less than 0.5 g/dL.

The levels of IL-6 on the 3rd day of fever was not different between the group with and without plasma leakage ($p>0.05$); while on the 5th day of fever, it was higher than the group with plasma leakage than the group without plasma leakage ($p<0.05$). CRP levels on the 3rd and 5th day were higher in the group with plasma leakage compared to the group without plasma leakage ($p<0.05$). (**Table 2**)

The levels of IL-6 in the groups with and without plasma leakage were higher on the 3rd day of fever than on the 5th day of fever in both groups ($p<0.05$). CRP levels were found higher on the 3rd day compared to the 5th day of fever in both groups ($p< 0.05$). (**Table 3**)

Table 2. Differences in interleukin-6 and CRP levels in groups of dengue infection with and without plasma leakage

Variables	With plasma leakage (n=24)	Without plasma leakage (n=20)	p*
IL-6 level (pg/mL), median (range)			
- The 3rd day	8.56 (1.85-96.15)	3.80 (1.94-81.93)	0.069*
- The 5th day	4.30 (1.60-70.28)	2.76 (1.26-11.67)	0.025*
CRP level (mg/L), median (range)			
- The 3rd day	10.1 (4.3-36.5)	6.8 (3.0-21.6)	0.014*
- The 5th day	5.0 (2.0-20.1)	2.9 (0.1-9.9)	0.048*

* Mann-Whitney test

Table 3. The levels of interleukin-6 and CRP on the 3rd and the 5th day of fever

Variables	On the 3rd day (n=44)	On the 5th day (n=44)	p*
IL-6 level (pg/mL), median (range)			
- With plasma leakage	8.56 (1.85-96.15)	4.30 (1.60-70.28)	0.037*
- Without plasma leakage	3.80 (1.94-81.93)	2.76 (1.26-11.67)	0.005*
CRP level (mg/L), median (range)			
- With plasma leakage	10.1 (4.3-36.5)	5.0 (2.0-20.1)	0.0001*
- Without plasma leakage	6.8 (0.3-21.6)	2.9 (0.1-9.9)	0.0001*

* Wilcoxon test

DISCUSSION

The mean age of subjects in both groups is still included in the group of young age, which is consistent with a study conducted by Bhaskar et al.[4] In few last decades, there are epidemiological changes of dengue infection in Indonesia, i.e. there are a higher number of dengue infection cases in the age group over 15 years of age.[15]

Similar data has also been shown by other Asian countries such as Singapore, which in 1990-1996 demonstrated the highest morbidity rate in the 15-34 years age group and Bangladesh which during the epidemic dengue in 2000 found the highest proportion cases in the 18-33 years age group.[18] The proportion between male and female subjects in our study is similar, which is consistent with reports from Malaysia and South America.[18]

The mean hemoglobin levels and hematocrit on the 3rd and 5th day in both groups were still within normal limit, which is consistent with the study conducted by Priyadarshini et al.[19] The absence of hemoconcentration in our study is probably due to fluid intervention during early treatment. Leukopenia has occurred on the 3rd day of fever in both groups.

In dengue infection, leukopenia usually occurs earlier than thrombocytopenia.[1] Thrombocytopenia had been identified on the 3rd day of fever in both groups and it was getting more severe on the 5th day of fever. Thrombocytopenia commonly occurs in DF and is always found in DHF/DSS.[1] In the group with plasma leakage, we found that the mean albumin levels on the 5th day of fever was lower than normal. It is consistent with the theory that plasma leakage of DHF occurs during the critical phase, which is characterized by albumin loss to the extravascular space due to increased capillary permeability.[20] It was found that the mean of delta albumin in the group with plasma leakage was more than 0.5 g/dL. It is consistent with the WHO criteria1 on the markers of plasma leakage in DHF patients.

In our study, there was no difference of IL-6 levels between both groups on the 3rd day of fever; while on the 5th day of fever, there was a higher levels of IL-6 in the group with plasma leakage compared to the group without plasma leakage. Various results of previous studies show different findings due to different range of study design, time set for sample collection and different population of patients and clinical classification. Priyadarshini et al[19] found higher IL-6 levels in DHF group compared to the DF group. Kumar et al[21] and Mangione et al[12] found that the IL-6 levels in the DHF group was not different from the levels in the DF group. Results of our study showed that IL-6 may have role in the development of plasma leakage in dengue infection. However, the effect of various other factors that has not been studies should also be considered including the cytokines and other inflammatory mediators, cellular and humoral immune factors, virus virulence and susceptibility of host genetic.[22]

The CRP levels on the 3rd and 5th day of fever was found higher in the group with plasma leakage compared to the group without plasma leakage. It indicates that there is more severe inflammatory process in the group with plasma leakage, either in the febrile or critical phase. However, differences in CRP levels between both groups on the 3rd day of fever were not accompanied by different IL-6 levels. It may occur since there is still an effect of TNF-α and IL-1β on CRP synthesis in the liver during the febrile phase.

The results of our study have confirmed the pathophysiology of the cytokine storm in dengue infection of adult patients. It is obvious that the cytokine storm reaction occurs in the febrile phase and it is resolved during the critical phase with an impact of plasma leakage in critical phase. According to Guabiraba et al.[23], there is cytokine storm with high levels of pro-inflammatory cytokines in DHF/DSS patients, which will be followed by activation of coagulation system, acute phase proteins, soluble receptor and other inflammatory mediators with end results of endothelial activation, vascular leakage, which are accompanied by bleeding and shock.[23]

Our study also found that IL-6 levels on the 3rd day was higher than on the 5th day of fever, both in the group with and without plasma leakage. It indicates that during the critical phase, the inflammatory process has been resolved in both groups. However, the IL-6 levels on the 5th day of fever was still higher in the group with plasma leakage compared to the group without plasma leakage. It demonstrates that IL-6 released during the febrile phase has equal strength in both groups, but the recovery process in the group with plasma leakage was slower; therefore, during the critical phase, we found

higher IL-6 levels compared to the group without plasma leakage. According to Chaturvedi et al.[5], if there is a shift of cytokine response to Th2 cytokines in dengue infection, which is characterized by a very high IL-6 production, then plasma leakage will occur.

Consistent with various previous studies, CRP levels were found higher on the 3rd day compared to the 5th day of fever in both groups. Nascimento et al.[24] reported that in the DFH and DF groups, there are higher levels of CRP than the healthy control. Kumar et al.[21] found that during the febrile phase, the CRP levels in DHF and DF groups are higher than the control group; while during the critical phase it is not different from the control. It indicates that there is more severe inflammatory process in dengue infection during the febrile phase compared to the critical phase.

CRP is one of acute phase proteins that will have increasing levels when inflammation occurs. In systemic viral infection, increased CRP levels may also occur.[13] During the febrile phase in dengue infection, there will be viremia that can induce acute inflammatory process, which is accompanied by increased CRP levels.[3,9] It is consistent with the basic function of CRP, i.e. to control inflammation, clearance stimulation of damaged cells and tissue component and to initiate the repair function.[25] Different CRP levels on the 3rd and 5th day of fever in both groups probably happened due to inflammatory reaction on the dengue virus itself, instead of secondary bacterial infection since we found reduced CRP levels on the 5th day of fever in both groups and during hospitalization, no subjects had received antibiotic treatment.

The limitation of our study that can affect the results is that we did not measure the levels of cytokines that induce synthesis of other CRPs, i.e. the TNF-α and IL-1β. In addition, the USG examination also did not measure other parameters for plasma leakage, which are thickening of gall bladder wall and pericardial effusion; therefore, there is a possibility of undetected plasma leakage in our study. Although we used secondary data, but there is small possibility of recall bias in our study since the subjects with incomplete data has been excluded from our study. The possibility of inaccurate measurement on duration of fever is also small because all of medical records in accessible population have been completed with CRF compiled by an experienced clinical team. Moreover, the USG examination to detect the presence of pleural effusion and ascites was performed by an experienced USG expert to avoid the inter-observer bias.

Results of our study can be applied in larger populations by evaluating generalizations. External validity of our study was relatively good since the subjects were recruited by consecutive sampling for approximately a year; therefore, we believe that it may represent all cases of dengue infection all year long. The second external validity of our study was also relatively good since the characteristics of patients with dengue infection who were hospitalized in both hospitals were not different from the dengue infection patients of other hospitals. Based on the above mentioned explanation, we suggest that the generalization of our study results can be done for adult patients with dengue infection who are hospitalized in all hospitals.

CONCLUSION

On the 3rd day of fever, the IL-6 levels were not different between the group of adult dengue infection patients with and without plasma leakage; while on the 5th of fever, the IL-6 levels were higher in the group with plasma leakage. The levels of IL-6 and CRP in both groups were higher on the 3rd day compared to the 5th day of fever. The clinical application of our study is that measurements of CRP levels can support the management of adult patients with dengue infection through increasing awareness on the development of plasma leakage.

REFERENCES

1. WHO. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition: World Health Organization, Regional Office for South-East Asia; 2011.
2. Brito CAA, Albuquerque MdFMP, Lucena-Silva N. Plasma leakage detection in severe dengue: when serum albumin quantification plays a role? Rev Soc Bras Med Trop. 2007;40(2):220-3.

3. TDR/WHO. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009.
4. Bhaskar ME, Moorthy S, Kumar NS, Arthur P. Dengue haemorrhagic fever among adults--an observational study in Chennai, South India. Indian J Med Res. 2010;132:738-40.
5. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol. 2000;28(3):183-8.
6. Dalrymple NA, Mackow ER. Endothelial cells elicit immune-enhancing responses to dengue virus infection. J Virol. 2012;86(12):6408-15.
7. Costa VV, Fagundes CT, Souza DG, Teixeira MM. Inflammatory and innate immune responses in dengue infection: protection versus disease induction. Am J Pathol. 2013;182(6):1950-61.
8. Basu A, Chaturvedi UC. Vascular endothelium: the battlefield of dengue viruses. FEMS Immunol Med Microbiol. 2008;53:287-299.
9. Juffrie M, Meer GM, Hack CE, et al. Inflammatory mediators in dengue virus infection in children: interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. Am J Trop Med Hyg. 2001;65(1):70-5.
10. Restrepo BN, Isaza DM, Salazar CL, Ramirez R, Ospina M, Alvarez LG. Serum levels of interleukin-6, tumor necrosis factor-alpha and interferon-gama in infants with and without dengue. Rev Soc Bras Med Trop. 2008;41(1):6-10.
11. Suharti C, van Gorp ECM, Dolmans WMV, et al. Cytokine patterns during dengue shock syndrome. Eur Cytokine Net. 2003;14(3):172-7.
12. Mangione JN, Huy NT, Lan NT, et al. The association of cytokines with severe dengue in children. Trop Med Health. 2014;42(4):137-44.
13. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev. 2006;24(1):33-50.
14. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41(2):580-637.
15. Citraresmi E, Hadinegoro SR, Akib AA. Diagnosis dan tata laksana demam berdarah dengue pada kejadian luar biasa tahun 2004 di enam rumah sakit di Jakarta. Sari Pediatri. 2007;8(3):8-14.
16. Van de Weg CA, Pannuti CS, de Araujo ES, et al. Microbial translocation is associated with extensive immune activation in dengue virus infected patients with severe disease. PLoS Negl Trop Dis. 2013;7(5):e2236.
17. Suwandono A, Kosasih H, Nurhayati, et al. Four dengue virus serotypes found circulating during an outbreak of dengue fever and dengue haemorrhagic fever in Jakarta, Indonesia, during 2004. Trans R Soc Trop Med Hyg. 2006;100(9):855-62.
18. Guha-Sapir D, Schimmer B. Dengue fever: new paradigms for a changing epide-miology. Emerg Themes Epidemiol. 2005;2(1):1.
19. Priyadarshini D, Gadia RR, Tripathy A, et al. Clinical findings and pro-inflammatory cytokines in dengue patients in Western India: a facility-based study. PLoS One. 2010;5(1):e8709.
20. Srikiatkhachorn A. Plasma leakage in dengue haemorrhagic fever. Thromb Haemost. 2009; 102(6):1042-9.
21. Kumar Y, Liang C, Bo Z, Rajapakse JC, Ooi EE, Tannenbaum SR. Serum proteome and cytokine analysis in a longitudinal cohort of adults with primary dengue infection reveals predictive markers of DHF. PLoS Negl Trop Dis. 2012;6(11):e1887.
22. Whitehorn J, Simmons CP. The pathogenesis of dengue. Vaccine. 2011;29(42):7221-8.
23. Guabiraba R, Ryffel B. Dengue virus infection: current concepts in immune mecha-nisms and lessons from murine models. Immunol. 2014;141(2):143-56.
24. Nascimento EJ, Silva AM, Cordeiro MT, et al. Alternative complement pathway deregulation is correlated with dengue severity. PLoS One. 2009; 4(8):e6782.
25. Biro A, Rovo Z, Papp D, et al. Studies on the interactions between C-reactive protein and complement proteins. Immunol. 2007;121(1):40-50.