Correlation of enhancement Mean Platelet Volume (MPV) with severity of dengue infection

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Abstract. Dengue infection is one of the infectious diseases which become a health problem in the world. Platelets play an important role in the pathogenesis of dengue. The quantity of platelet related to dengue were often discussed but the quality of platelet related to dengue infection were rarely discussed. The activation of platelets can be observe indirectly through changes in MPV. Aim of this study is to analyze the prevalence ratio of MPV enhancement in dengue hemorrhagic fever (DHF) compared to dengue fever (DF). This study is an observational analytical study with a cross-sectional design to analyze the prevalence ratio of enhancement MPV in DHF compared to DF. The subjects were dengue suspected patients that hospitalized in Dr. Sardjito General Hospital and PKU Muhammadiyah Hospital Yogyakarta who meet the inclusion and exclusion criteria. Venous blood were collected into 2 mL EDTA tube for automatical hematology analysis. Chi-square was used to analyze different proportions of MPV enhancement between DHF and DF groups. One hundred subjects were participated in this study and classified into DF and DHF based on WHO 2011 criteria. Prevalence ratio of MPV enhancement ≥9.7 fL was 4.8 (95% CI 2.8-37.0), p<0.0001. The result indicates that MPV enhancement was a risk factor of worsening dengue infection.

1. Introduction
According to the 2011 World Health Organization (WHO) data, DF/DHF incidence rate has been increasing exponentially every 10 years. The average annual cases number in 2000 to 2008 was 1,686,870, or nearly three and a half times the number obtained between 1990 and 1999 which was 479,848 [1]. In 2012, the number of dengue hemorrhagic fever (DHF) patients in Indonesia were as many as 90,245 cases with 816 deaths (Incidence Rate (IR) 37.11 per 100,000 population and Case Fatality Rate (CFR) 0.90%). There was increasing number of cases in 2012 than in 2011, in which 65,725 cases with morbidity 27.67 were reported. In line with increasing IR, the number of districts/cities affected by dengue in 2012 also increased, from 374 (75.25%) districts into 417 districts/cities (83.9%) in 2012. This increase indicates the extent of the spread of dengue disease [2].

One aspect that is implicated in the pathogenesis of dengue is platelets. To date, the emphasis on platelets in only limited to platelet count, while quality of platelets (both activation and dysfunction) are rarely discussed. Adoption of a parameter to determine platelet dysfunction or activation such as β-thromboglobulin, platelet factor 4, P-selection expression, or angiopoietin are still relatively expensive
and their use in hospital services is very limited. ADVIA 120 hematology system is an instrument that is able to measure MPV (one part of platelet indices) which may indirectly depict platelet activation and function. Some platelet indices are already known by clinicians as an indicator of platelet activation [3]. Mean Platelet Volume describes the platelet function and activity. Higher MPV indicates a higher large platelet count which is a sign of increasing platelet turnover [4].

The role of platelet indices as predicting factors of dengue infection severity has not been much discussed. A previous study suggested that there were significant differences in MPV between sepsis and severe sepsis groups. Median of MPV in severe sepsis group (8.00 fl) were significantly higher than sepsis group (7.00 fl) [5]. These results indicated that we cannot rule out the possibility of MPV as predictor’s dengue infection through prevalence ratio analysis. The current study tried to analyze the prevalence ratio of enhancement MPV on the incidence of DHF compared with DF.

2. Material and methods

This is an observational analytic study with cross sectional design to determine the prevalence ratio of MPV enhancement in DHF groups compared to DF groups. Subjects were consecutive dengue patients hospitalized in Dr. Sardjito Hospital and PKU Hospital Yogyakarta and fulfilled the inclusion and exclusion criteria. Inclusion criteria were dengue infection patients, who based on their clinical symptoms diagnosed as probable dengue by clinicians, aged ≥10 years, in defervescence phase, and serological tests showed NS 1 positive, anti-dengue IgM positive and anti-dengue IgG positive, or anti-dengue IgG positive and anti-dengue IgG negative. Exclusion criteria were if the sample was not feasible (lysis, clot, less volume, non-EDTA anticoagulants), missing data (refusal of hospital treatment by patient), coinfection with other acute infectious diseases (typhoid fever, malaria and chikungunya), history of hematological malignancy, diabetes mellitus, stroke, and acute coronary syndrome based on history, communication with clinicians, questionnaires, and medical records. In this study, subjects were divided into groups DD and DHF according to WHO criteria 2011. The value of MPV in this study were categorized into “increased” group if the value was exceeds above the upper limit of the normal range (≥9.7 fl for MPV).

The study was conducted at Dr. Sardjito Hospital and PKU Muhammadiyah Hospital Yogyakarta for approximately five (5) months between November 2014 and March 2015. Routine blood tests, including platelets indices were performed at Clinical Laboratory Department of Dr. Sardjito Hospital Yogyakarta. Subjects’ history and physical tests were conducted by the residents, internal medicine specialist or pediatrician. Dengue serological tests, routine blood tests and platelet indices were performed at Clinical Laboratory Department of Dr. Sardjito Hospital Yogyakarta. Mean Platelet Volume test were performed using venous blood samples (median cubital vein) treated with 2 mL EDTA anticoagulant and performed using ADVIA 120 Hematology System. Tests were performed immediately after sampling, with a maximum time gap of 4 hours after sampling.

Variables analyzed in the study include subject characteristics, clinical and laboratory data. Subjects characteristics include age, gender, number of fever days at sampling. Laboratory data were dengue serology, hematocrit value, leukocyte count, platelet count, and MPV. Analysis began with the analytical performance test (calibration, accuracy, and precision test so that the results are valid and reliable). Patients who met the inclusion and exclusion criteria then tested for automatical hematology analysis, including MPV.

Subjects/subjects’ parents who were willing to participate in the study were required to sign an informed consent. This study had been approved by Medical and Health Research Ethics, Faculty of Medicine, University Gajah Mada. The collected data is checked for completeness and accuracy, then coded, tabulated, and entered into database. Data characteristics were presented in a descriptive manner. Test of difference were conducted on platelet indices in DF and DHF group using Independent t-test or Mann-Whitney test. Categorical data were presented as proportion. Proportion were analyzed with Chi-square by calculating the prevalence ratio with 95% confidence interval, p <0.05. The entire calculation was done with computerized systems.
3. Results and discussion
In total, 100 subjects met the inclusion and exclusion criteria, divided into 2 groups: 52 subjects in the dengue fever (DF) group and 48 subjects in the dengue hemorrhagic fever (DHF) group. Data in table 1 suggested that there were significant differences in platelet count, hematocrit, and MPV between DF and DHF groups.

Data in table 1 suggested that mean value of MPV in DF and DHF groups were above the normal range for adult MPV (6.7 – 9.6 fL) [6]. This is consistent with a study which suggested that patients with more severe dengue infection had significantly higher MPV [7].

| Variable         | DF        | DHF       | p       |
|------------------|-----------|-----------|---------|
| Platelet (10^3/μL) | 28 - 147  | 10 - 70   | <0.0001*|
| Median (min–max)  |           |           |         |
| Leukocyte (10^3/μL) | 3.63 (0.91–9.59) | 3.87 (0.96–9.64) | 0.165* |
| Median (min–max)  |           |           |         |
| Hematocrit (%)    | 39.1 ± 4.2 | 41.8 ± 4.1 | 0.002** |
| MPV (fL)          | 10.4 ± 1.20 | 11.18 ± 1.30 | 0.003*  |
| Mean ± SD         |           |           |         |

Description:
* Mann Whitney U test
** Independent t test

Based on the table 2, the majority of DHF patients were classified into “increased MPV”, indicates that there is more platelet activation in DHF patients. It was suggested that endothelial damage may induce platelet activation and adhesion to endothelium [8]. This may explain the high percentage of DHF patients with high MPV, since there is increased vascular permeability in DHF patients [8].

| Severity | DHF n (%) | DF n (%) | Total |
|----------|-----------|----------|-------|
| MPV      |           |          |       |
| Increased (≥9.7 fL) | 45 (93.8)  | 31 (59.9) | 76    |
| Not increased (<9.7 fL) | 3 (6.2)    | 21 (40.4) | 24    |
| Total    | 48        | 52       | 100   |

There was significant prevalence ratio for increased MPV. In the current study, the prevalence ratio of increased MPV was 4.8, 95% CI 2.8 - 37.0, p <0.001, which means that the higher MPV (≥9.7 fL) was significantly 4.8 times more common in DHF patients than DF. In the sample population studied, we believe the 95% prevalence ratio was between 2.8 - 37.0, thus increased MPV can be considered as a good marker for worsening dengue infection.

So far, there has been no previous studies that investigate prevalence ratio of increased MPV in dengue patients. Several previous studies regarding dengue infection had been studying the correlation between MPV with platelet count. Low platelet count is a reflection of worsening dengue infection [7,9-11]. Gunawan et al found a negative correlation between MPV with platelet count (r = -0.52; p <0.05) [7]. Based on these results it can be suggested that the more severe dengue infection (more thrombocytopenic) will be positively correlated with higher MPV, thus it can be said that the study by Gunawan et al seems consistent with the results of the current study.

Platelet indices was negative correlated with platelet count (MPV with r = -0.805) in the case of increased platelet destruction such as immune thrombocytopenic purpura, dengue, malaria, and chronic liver disease [12]. Increased platelet destruction may induce a state of thrombocytopenia [13,14].
According Maedel et al, thrombocytopenia in turn induce the bone marrow to produce immature platelets with larger size, increasing MPV value [15]. In dengue infection there is a state of thrombocytopenia primarily due to increased peripheral destruction which may induce the bone marrow to release immature platelets with larger size into the peripheral circulation. Mean Platelet Volume describes the level of platelet stimulation or production [16].

The advantage of this study were using of automated hematology instruments that have been commonly used in the measurement of complete blood count and also the results of the prevalence ratio was > 1 (p < 0.05) so it can be applicable. The limitations of this study is the result of 95% CI in increase MPV group has width interval that may be caused by difficulty of determining a subject that was really in defervescence phase (free of fever phase without the influence of drugs to decrease fever). In addition the design of this research is still in the form of cross-sectional design so it cannot explain the temporal relationship (causality) so it is advisable for the development of further research to be done with a cohort design so it can find the value of the relative risk which can explain the dynamics of temporal relationship (causality).

4. Conclusion
The prevalence ratio of increased MPV were significantly 4.8 times in DHF population than DF population. The result indicates that MPV enhancement was a risk factor of worsening dengue infection. Further studies with cohort design are needed to investigate increased MPV to assess the worsening dengue infection.

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