Correlation of enhancement Platelet Distribution Width (PDW) with severity of dengue infection

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Abstract. Dengue infections are currently one of the most rapidly emerging arboviral infections in the world. During this time the emphasis of platelets only limited about number of platelets and very rarely discuss about the quality of platelets (activation and dysfunction). Changes in PDW reflects the activation of platelets. Aim of this study is to analyze the prevalence ratio of PDW enhancement in dengue hemorrhagic fever (DHF) compared to dengue fever (DF). This was a cross-sectional study to analyze the prevalence ratio of enhancement PDW in DHF compared to DF. The subjects were dengue suspected patients who meet the inclusion and exclusion criteria. Venous blood were collected for automatical hematology analysis (include PDW). Different proportions of PDW enhancement between DHF and DF groups was analyzed by Chi-square. One hundred subjects were participated in this study and classified into DF and DHF. Prevalence ratio of PDW enhancement (≥64.8%) was 3.1 (95% CI 1.5 – 20.7), p 0.006. The result indicates that PDW enhancement was a risk factor of worsening dengue infection.

1. Introduction
Dengue is a systemic viral disease, transmitted to humans by the bite of infected Aedes spp. mosquitoes throughout the tropical and subtropical world. It results in substantial disease burden, health service disruption and massive socioeconomic losses. [1-6] It is estimated globally that 50 to 100 million dengue cases occur each year across approximately half of the world’s population, especially in areas with cocirculation of multiple virus serotypes, known as hyperendemic regions in Southeast Asia and Pacific. [1,7-8] It is also estimated that dengue is responsible for 20,000 deaths annually. [7-9] A study by Bhatt et al estimated that dengue infection cases have increased more than 3 times per year with 67 to 136 million cases annually which manifest clinically at any level of severity [10].

Severity of the illness is determined by various risk factors such as age, pre-existing illness, infecting serotype, and secondary infection. A second infection with a different serotype leads to more severe form of the disease than the primary infection. [11] One of the most common laboratory findings in dengue is thrombocytopenia. [12] The complex mechanism of thrombocytopenia remains unclear. Possible mechanisms of thrombocytopenia could be, direct bone marrow suppression by the virus; anti-dengue antibody-mediated platelet destruction, peripheral consumption of platelets and
isolated viral replication in the platelet. Thrombocytopenia leads to bleeding although the platelet count may not directly correlate with the bleeding manifestation. [13] Recently, novel platelet indices have been investigated as prospective platelet activation markers. [14] Platelet Distribution Width is one part of platelet indices, it indicates platelets anisocytosis level. Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW) which increases during platelet activation [14].

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 PDW between sepsis and severe sepsis groups. Median of PDW in severe sepsis group (18.0%) were significantly higher than sepsis group (17.0%). Severe sepsis patients who have greater than 18% PDW levels have a higher risk of death. [15] These results indicated that we can not rule out the possibility of PDW as predictors 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. Methods
This is an observational analytic study with cross sectional design to determine the prevalence ratio of PDW enhancement in DHF groups compared to DF groups. Subjects were consecutive dengue patients hospitalized in Dr. Sardjito Hospital and PKU Hospital Yogyakarta who met 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 IgM 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 PDW in this study were categorized into “increased” group if the value was exceeds above the upper limit of the normal range (≥54.8% for PDW).

The study was conducted at Dr. Sardjito Hospital and PKU Muhammadiyah Hospital Yogyakarta for approximately five (5) months between November 2014 and March 2015. Complete blood count, 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 paediatrician. Dengue serological tests, complete blood count and platelet indices were performed at Clinical Laboratory Department of Dr. Sardjito Hospital Yogyakarta. Platelet Distribution Width test were performed using 2 mL venous blood samples (median cubital vein) treated with EDTA anticoagulant and performed using ADVIA 120 Haematology 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, haematocrit value, leukocyte count, platelet count, and PDW. Patients who met the inclusion and exclusion criteria then tested for routine blood, including platelet indices.

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 and tabulated and entered into database. Data characteristics were presented in a descriptive manner. Test of difference were conducted on PDW in DF and DHF group using Independent t-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
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, haematocrit, and PDW between DF and DHF groups.

Data in table 1 suggested that mean value of PDW in DF and DHF groups were above the normal range for adult PDW (39.3 – 64.7%). [16] This is consistent with a study by Gunawan and colleagues (2010) which suggested that patients with more severe dengue infection had significantly higher PDW [17].

| Variable                  | Dengue Fever | Dengue Haemorrhagic Fever | p       |
|---------------------------|--------------|---------------------------|---------|
| Age (years)               |              |                           |         |
| Median (min – max)        | 22 (10 – 68) | 19.5 (10 – 50)            | 0.323*  |
| <18 years                 | 10 (19.2%)   | 18 (37.5%)                |         |
| ≥18 years                 | 42 (80.8%)   | 30 (62.5%)                |         |
| Gender M/F                | 25/27        | 27/21                     | 0.414** |
| Fever duration (days)     | 5 (3 – 8)    | 5 (3 – 8)                 | 0.778*  |
| Platelet (10³/ L)         | 28 - 147     | 10 - 70                   | <0.0001*|
| Leukocyte (10³/ L)        | 3.63 (0.91 – 9.59) | 3.87 (0.96 – 9.64) | 0.165*  |
| Hematocrit (%)            |              |                           |         |
| Mean ± SD                 | 39.1 ± 4.2   | 41.8 ± 4.1                | 0.002***|
| PDW (%)                   | 69.06 ± 6.66 | 74.68 ± 7.54              | <0.0001***|

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

Based on the table 2, the majority of DHF patients were classified into “increased PDW”, indicates that there is more platelet activation in DHF patients. Levi and Poll (2013) suggested that endothelial damage may induce platelet activation and adhesion to endothelium. This may explain the high percentage of DHF patients with high PDW, since there is increased vascular permeability in DHF patients [18].

| Severity                  | DHF n (%)  | DF n (%)  | Total |
|---------------------------|------------|-----------|-------|
| PDW Increased (≥64.8%)    | 45 (93.8)  | 38 (73.1) | 83    |
| Not increased (<64.8%)    | 3 (6.2)    | 14 (26.9) | 17    |
| Total                     | 48         | 52        | 100   |

There was significant prevalence ratio for increased PDW. In the current study, the prevalence ratio of increased PDW was 3.1, 95% CI 1.5 – 20.7, with p value 0.006, which means that the higher PDW (≥64.8%) was significantly 3.1 times more common in DHF patients than DF. In the sample population studied, we believe the 95% prevalence ratio was between 1.5 – 20.7, thus increased PDW can be considered as a good marker for the development of DHF (worsening dengue infection).
4. Discussion
So far there has been no previous studies that investigate prevalence ratio of increased PDW in dengue patients. Several previous studies regarding dengue infection had been studying the correlation between PDW with platelet count. Platelet count is a reflection of worsening dengue infection. Gunawan and colleagues (2010) found a negative correlation between PDW with platelet count \((r = -0.77; p <0.05)\). [17] Based on these results it can be suggested that the more severe dengue infection (more thrombocytopenic) will be positively correlated with higher PDW, thus it can be said that the study by Gunawan and colleagues (2010) seems consistent with the results of the current study. Platelet indices was negative correlated with platelet count \((PDW) with r = -0.996\) in the case of increased platelet destruction such as immune thrombocytopenic purpura, dengue, malaria, and chronic liver disease [19].

Platelet distribution width measures variations in platelets size circulating in the peripheral blood, younger platelets are larger and older platelets have smaller size. In dengue infections, peripheral platelet destruction increase in which the bone marrow will try to compensate by releasing younger or immature platelets. Thus the platelets in circulation of dengue patients are biphasic, younger platelets with larger size and older platelets with smaller size. In general, the smaller sized platelets have a lower functional capacity than large-sized platelets thus increasing the risk of bleeding [17].

The advantage of this study were using of automated haematology 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 PDW group has width interval that may be caused by difficulty of determining a subject that was really in deverfescence 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 can not 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).

5. Conclusion
The prevalence ratio of increased PDW were significantly 3.1 times in DHF population than DF population. The result indicates that PDW enhancement was a risk factor of worsening dengue infection. Further studies with cohort design are needed to investigate increased PDW to assess the worsening dengue infection.

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