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Peripheral blood examination to assess bleeding risk in children with dengue infections

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Abstract

Background Dengue viral infection may cause mild to severe clinical manifestations, with or without bleeding. A number of factors may cause bleeding in patients with dengue. However, health providers may be unable to perform the examinations required to sufficiently predict the risk of bleeding.

Objective To find risk factors for bleeding using peripheral blood examinations in children with dengue infection.

Methods This cross-sectional study was conducted at the Pediatric Ward of the Dr. Saiful Anwar General Hospital, Malang, from January 2010 to December 2011. We included children aged 1 to 18 years with dengue viral infection, as confirmed by the 1997 WHO criteria and serology. Peripheral blood examinations were made daily, depending on the patient's condition. We classified the bleeding status into non-bleeding, petechial bleeding (mild hemorrhage), and mucosal bleeding (severe hemorrhage). We recorded subjects' bleeding status at the time of their highest packed cell volume (PCV), and recorded their leukocyte and platelet counts at that time. We computed the parameters' medians and compared them to bleeding status by Chi-square test. For significant ($P<0.05$) associations we calculated the OR (odds ratio) with a 95% confidence interval. All patients were treated according to the 1997 WHO dengue guidelines.

Results There were all 294 subjects with dengue and 282 subjects had complete data, 202 with bleeding (120 petechial, 82 mucosal bleeding) and 80 without bleeding. The median PCV was 36.8%, while median platelet count was 51,000/μL and median leukocyte count was 3,400/μL. The OR of PCV > 36.8% for bleeding was 2.31 (95%CI 1.35 to 3.95). The OR of platelet count <51,000/ μL for bleeding was 2.34 (95%CI 1.37 to 3.99) compared to platelet count > 51,000/ μL. The OR of platelet count < 51,000/ μL for mucosal bleeding was 3.39 (95%CI 1.78 to 6.48). Chi-square analysis for leukocyte count showed it was not associated with bleeding in dengue ($P= 0.186$).

Conclusion The PCV level > 36.8% increased the risk for bleeding by 2.31 times, for both petechial and mucosal bleeding. Platelet count < 51,000/ μL increased the risk for bleeding 2.34 times and for mucosal bleeding by 3.4 times. Leukocytes count was not associated with bleeding. Basic laboratory examinations of PCV and platelet count may, therefore, be used as a predictor of bleeding in children with dengue infection. **[Paediatr Indones. 2012;52:175-80]**.

Keywords: *peripheral blood, dengue virus, bleeding*

Dengue viral infection is the most prevalent arboviral disease worldwide. It is caused by infection with any one of four dengue virus (DENV) serotypes (DENV1-4). DENV infections cause illness in tens of millions each year throughout the tropics and subtropics and severe morbidity in approximately 2 million persons/year, with approximately 20,000 deaths/year.[1] In Indonesia,

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dengue viral infections have spread through all provinces and villages since 1993, with the highest incidence in 1998 with about 72,133 patients.[2]

Dengue virus infections may be subclinical or manifest as dengue fever (DF) or dengue hemorrhagic fever (DHF). DHF is a syndrome with 3–7 days of fever, headache, myalgia, and rash, accompanied by leukopenia and varying degrees of thrombocytopenia.[3] An early clinical diagnosis of DHF is difficult because the World Health Organization (WHO) clinical and laboratory criteria for DHF may be manifested only in the late phase of acute illness.[4] The latest WHO criteria (2009) reported mucosal bleeding as a warning sign of dengue, leading to severe dengue when it presents as severe bleeding.

The pathogenesis of bleeding in dengue viral infection encompasses 3 mechanisms: vasculopathy, thrombopathy, and coagulopathy. These 3 mechanisms are caused by interconnected complement activation cascades.[3-5] Measuring vasculopathy, thrombopathy, and coagulopathy is complex, expensive, and cannot be done in all health care settings. The routine follow-up for dengue patients includes a simple and low-cost complete blood examination, especially in the post-febrile defervesence period, which is a particularly critical phase. The aim of this study was to determine the predictive value of simple laboratory examination on bleeding tendency in dengue patients. The examinations include packed cell volume (PCV), leukocyte count, and platelet count, all of which are available in limited resource settings.

Methods

This cross-sectional study was undertaken from January 2010 to December 2012. We included all children (1 to 18 years old) admitted to Dr. Saiful Anwar General Hospital, with dengue viral infection only. The dengue diagnoses were made according to WHO 1997 criteria and confirmed by serology. As suggested in the guidelines, hematologic examinations were performed daily, depending on patients' clinical condition, including PCV levels, platelet counts, and leukocyte counts. The highest median PCV was recorded with the assumption that it represented the critical phase (leakage period). Median leukocyte counts and platelet counts were also taken at that time. We did not measure or observe bleeding volumes nor outcomes of the patients. Data of patients requiring blood or other transfusion components was taken only up to the time of transfusion.

Laboratory examinations were performed in the Clinical Pathology Laboratory of Dr. Saiful Anwar General Hospital. We did not collect informed consent in this study because the laboratory examination was part of the dengue management.

Subjects with bleeding were divided into 2 groups, i.e. mild bleeding represented by petechial bleeding, and mucosal bleeding with the potential for severe bleeding. The WHO 2009 criteria reported mucosal bleeding to be a warning sign which may lead to severe dengue. However, in the WHO 1997 criteria, a spontaneous bleeding tendency was defined as dengue hemorrhagic fever grade II. The bleeding tendency of each patient was recorded at the same time the highest PCV data was taken.

We calculated the odds ratio (OR) of the PCV level, leukocyte count, and platelet count relationships to non-bleeding, petechial bleeding, and mucosal bleeding case status.

Results

There were 294 patients with dengue viral infection, but only 282 had complete data. Among these 282, 202 had bleeding (120 petechial bleeding, 82 mucosal bleeding) while 80 had no bleeding. The median PCV was 36.8%, median platelets count was 51,000/μL and median leukocytes count was 3,400/μL. The numbers of male and female patients with dengue viral infection were not significantly different. The incidence of petechial bleeding appeared greater than mucosal bleeding in each of the above blood parameter groups. The basic characteristics of subjects are shown in Table 1.

Table 2 shows the OR of predicting the risk of bleeding. Subjects with a PCV >36.8% had 2.31 times the risk of bleeding than those with PCV < 36.8%. The leukocytes count was not significantly associated with bleeding tendency. Subjects with platelets count < 51,000/μL had 3.4 times the risk for mucosal bleeding than those with platelet count > 51,000/μL.

Table 1. Subjects' basic characteristics

Characteristics	Non-bleeding (n=80)	Petechial bleeding (n=120)	Mucosal bleeding (n=82)	Total (n=282)
Male	46	63	44	153
Female	34	57	38	129
Median PCV				36.8%
Median leucocytes count				3,400/ µL
Median platelets count				51,000/ µL
PCV < 36.8%	52	53	37	142
PCV > 36.8%	28	67	45	140
Leucocytes count < 3,400/µL	46	53	44	143
Leucocytes count >3,400/µL	34	67	38	139
Platelets count < 51,000/µL	30	63	55	148
Platelets count >51,000/µL	50	57	27	134
Stage of dengue hemorrhagic feve				124
DHF I				32
DHF II				36
DHF III				61
DHF IV				29

Table 2. Association of bleeding to PCV, leukocytes count, and platelets count

	Non-bleeding n=80	Bleeding n=202	OR (95% CI)	Non-bleeding n=80	Petechial bleeding n=120	OR	Non-bleeding n=80	Mucosal bleeding n=82	OR (95% CI)
PCV, n									
> 36.8%	28	112	2.31	28	67	2.35	28	45	2.26
<36.7%	52	90	(1.35 to 3.95)	52	53	(1.31 to 4.21)	52	37	(1.20 to 4.25)
Leukocyte count, n									
< 3,400/µL	46	97		46	53		46	44	
>3,400/µL	34	105		34	67		34	38	
Chi-square P=0.128									
Platelet count, n						1.84			
< 51,000/µL	30	118	2.34	30	63	(1.03 to 3.28)	30	55	3.4
>51,000/µL	50	84	(1.37 to 3.99)	50	57		50	27	(1.78 to 6.48)

Discussion

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcomes. While most patients recover following a self-limiting, non-severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without hemorrhage.[6] The WHO 1997 criteria guideline groups symptomatic dengue virus infections into three categories: undifferentiated fever, dengue fever (DF) and dengue hemorrhagic fever (DHF). DHF is further classified into four severity grades, with grades III and IV being defined as dengue shock syndrome.[7]

There are several difficulties in applying these criteria in that some cases cannot be classified into one of those three dengue categories. The WHO 2009 criteria guideline is more practical for clinicians. The WHO 2009 criteria classify dengue cases into three groups. Group (1) comprises probable dengue cases, defined as a person who traveled or lived in an endemic area, and had fever with 2 of following criteria: nausea,

vomiting, rash, aches and pain, positive tourniquet test, leukopenia, or any warning signs. Group (2) comprises dengue cases with warning signs, such as abdominal pain and tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, liver enlargement > 2 cm, and increasing PCV concurrent with rapidly decreasing platelet count. Group (3) comprises severe dengue, defined as cases with severe plasma leakage (leading to shock or fluid accumulation with respiratory distress), severe bleeding (evaluated by clinician), and severe organ involvement (liver: AST > 1000; brain: decreased level of consciousness, heart and/or other organs).[6,8] These criteria require a basic and simple hematologic examination to classify each case as probable dengue with or without warning signs. The hematological exam should include hemoglobin (Hb) level, PCV, leukocyte and platelet counts. Monitoring for bleeding is required in dengue cases with warning signs that require hospital admission.

We observed that patients with PCV > 36.8% had a 2.31 times greater risk for bleeding than those with PCV < 36.8%. We used the highest PCV value for each subject in our calculations in this study with the assumption that patients were in the critical phase at that time. The critical phase is characterized by increasing PCV concurrent with decreasing platelet count. Although a bleeding tendency usually occurs as a manifestation of plasma leakage and shock,it may occur in cases without plasma leakage (previously classified as dengue fever), usually manifested as thrombocytopenia.[6,9,10] Among the bleeding cases, there was no statistical evidence that PCV > 36.8% was a potential risk factor for mucosal bleeding, only compared to petechial bleeding.

Increased PCV represents the hemoconcentration in dengue, which is caused by vascular leakage. One possible cause of vascular leakage is platelet activating factor (PAF), which can produce many features of systemic inflammatory response syndrome (SIRS), such as hypotension, increased vascular permeability, cytokine release, and shock.[5,11,12]

Thrombocytopenia and plasma leakage are the main characteristics of severe dengue disease. It has been postulated that autoimmunity is involved in dengue pathogenesis, especially through the generation of autoantibodies against platelets, endothelial cells, and coagulatory products. The cross-reactive antibodies may cause platelet dysfunction, endothelial cell damage, coagulation defects, and macrophage activation.[13]

The platelets count and the platelets usage for sealing the vascular breakage may be considered as the cause of bleeding.[9] The OR of platelets in non-bleeding and petechial cases (mild bleeding) was only 2.34, indicating that subjects with platelet count < 51,000/μL only had 2.34 times greater risk for bleeding (both petechial and mucosal) than subjects with platelet count > 51,000/μL. Subjects with a platelets count < 51,000/μL had a 3.4 times greater risk of having mucosal bleeding than those with > 51,000/μL, and subjects with a platelets count < 51,000/μL had a 1.84 times greater risk of having petechial bleeding than those with > 51,000/μL. This observation supports the notion that petechial bleeding may be considered as a milder and less dangerous bleeding tendency than mucosal bleeding. However, we did not evaluate other parameters to assess bleeding tendency in this study, such as hemostatic states by activated partial thromboplastin time (APTT) and PPT.

We observed several cases (n=55; 67%) with platelet count < 51,000/μL with severe hemorrhage (mucosal bleeding). This hemorrhaging may be caused by a thrombopathy (platelet dysfunction), in addition to the decrease of platelets in peripheral blood. Thus, the decrease in platelets count (thrombocytopenia) may not be the only cause of bleeding, but may be one risk factor for bleeding tendency during dengue infection.

The bleeding tendency in DHF/DSS patients involves the role of both vascular endothelial cells and platelets, although the pathogenic mechanism is not fully understood. Studies on other viruses such as CMV, HIV, and hepatitis C virus has shown the presentation of platelet autoantibodies to be the cause of thrombocytopenia. In dengue viral infection these auto anti-platelet antibodies induce complement-mediated cell lysis, which may play role in thrombocytopenia. During blood vessel injury, activated platelets adhere to the injury site, aggregating together through fibrin formation.[3,14] Carlos *et al* concluded that thrombocytopenia was not the only cause of bleeding in dengue, but that the prolongation of APTT and reduction in fibrinogen concentration were strongly associated with severity of vascular leakage, leading to hypotension, shock

and finally bleeding.[3] Other studies have reported that viremia may play a role in determining clinical parameters, as is the case of secondary infection. Higher viral load may be used as an independent predictor for more severe thrombocytopenia, but may also be associated with a small increase in hemoconcentration.[15-17] Examinations to determine viral load are expensive and not available in all settings.

The leukocyte count of our subjects was not significantly associated with a bleeding tendency. Hence, it should not be considered as a risk factor for predicting bleeding. However, several studies have reported contrasting result on leukocyte counts in dengue. A Thai study showed that leukopenia was a good predictor of dengue infection in children, whilea study in Nicaragua stated that leukopenia was significantly associated with dengue in adults, but not in children.A study in Puerto Rico was inconclusive about the role of leukopenia in dengue, but their results showed that leukocyte counts in dengue cases were lower (mean 6900/μL) than those of non-dengue cases.[8,18-20] Another study on risk factors for DSS in children concluded that leukopenia < 4000/μL was a significant risk factor for shock in dengue. However, the authors did not mention an association between leukopenia and hemorrhage.[21]

Subjects with PCV levels of higher than 36.8% had a 2.31 times higher risk for bleeding, either petechial or mucosal. Furthermore, a platelet count of less than 51,000/μL showed a 3.4 times higher risk for mucosal bleeding. Leukocyte count was notsignificantly correlated to bleeding in our dengue cases. As such, a basic laboratory examination of PCV and platelet count may be used as a predictor of bleeding in pediatric dengue cases.

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