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Original Article

# Plasma prothrombin time and activated partial thromboplastin time as predictors of bleeding manifestations during dengue hemorrhagic fever

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## Abstract

*Background* Massive bleeding and shock are complications of dengue hemorrhagic fever (DHF) that are associated with high mortality. Impaired hemostasis, especially coagulopathy, contributes to bleeding manifestations in DHF. Parameters such as activated partial thromboplastin time (APTT) and plasma prothrombin time (PPT) indicate the impact of coagulation system.

***Objective*** To determine the relationship between APTT and PPT levels with bleeding manifestations in DHF patients.

***Methods*** A prospective cohort study was applied to subjects diagnosed with DHF at the Infection and Tropical Diseases Division, Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Denpasar, Indonesia. Laboratory tests to determine APTT and PPT were carried out on the third, fourth, and fifth day after the onset of fever. Bleeding manifestations were examined in patients during their hospital stay. Univariate and Cox regression analyses were performed to examine relationship between APTT and PPT values with bleeding manifestations in DHF patients.

***Results*** Forty-three children were enrolled in this study. There was a significant relationship between increases in APTT value with bleeding manifestations in DHF patients [RR 2.79 (95%CI 1.68 to 4.69), P <0.01]. Cox regression analysis showed that only increased APTT values correlated with bleeding manifestations [RR 2.05 (95%CI 1.92 to 3.90), P = 0.02].

***Conclusion*** APTT values may be used as a predictor for bleeding manifestations in DHF. **[Paediatr Indones. 2009;49:69-74]**.

***Keywords***: *DHF, impaired coagulation, bleeding, APTT*

In the last decade, the incidence of dengue viral infection has increased dramatically, and the disease is becoming more severe. Dengue viral infection can be fatal. The World Health Organization (WHO) reported that dengue hemorrhagic fever (DHF) incidence was 30,000 cases per year in the 1960s and that this has increased to 20 million cases per year more recently.[1-3]

Massive bleeding and shock are the most severe complications of DHF, and these lead to high mortality. Even though in general the mortality of DHF has decreased to 1.9%, the mortality of dengue shock syndrome (DSS) remains high.[4] At Dr. Kariadi Hospital, Semarang, Indonesia, the mortality rate of severe DHF (DSS with massive bleeding, prolonged shock, or recurrent shock) was 51.2% in 1998 but this had decreased to 12% by 2002.[5] This condition was a result of difficulty in predicting the clinical course of DHF, especially in early prediction of shock and bleeding manifestations. One factor that contributes

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to delayed early detection of shock and bleeding is that no laboratory tests can be used as a predictor of bleeding manifestations in DHF.[4,5]

Impaired hemostasis and vascular leakage are believed to be the main pathogenesis and pathophysiology in DHF, leading to vascular and platelet disorders and coagulopathy.[6-8] There are numerous DHF studies, but studies on the complete range of clinical nature of DHF involving large number of patients have been scarce.[9,10] A study by Wills *et al*,[11] in Vietnam, showed that plasma prothrombin time (PPT) and activated partial thromboplastin time (APTT) examinations can be used to predict bleeding manifestations during DHF. This study aimed to determine the relationship between APTT and PPT with bleeding manifestations in subjects suffering from DHF.

## Methods

We conducted a prospective cohort study between March 1st 2007 and May 31st 2007. Subjects were DHF patients aged one to 12 years with fever for not more than five days, and who were hospitalized at the Infection and Tropical Diseases Division, Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Denpasar, Bali, Indonesia. The diagnosis of DHF was based on 1997 WHO criteria and confirmed with anti-dengue serologic examination. We excluded DHF patients with underlying hemostasis disease, those who were transferred to another hospital, or insisted on discharge before the evaluations were made.

Sample size was calculated to obtain 80% power with 5% significance level and bleeding proportion on APTT/PTT normal groups 0.15 (RR=2). Forty-three subjects were required as the minimal sample size for both the risk group and the control group. Subjects were obtained using consecutive sampling in each group. We obtained parental written informed consent from all participants. The study protocol was reviewed and approved by the Ethics Committee of Udayana University, Sanglah Hospital, Denpasar, Indonesia.

Blood specimens for PPT and APTT examinations were collected on the 3rd, 4th, or 5th day of fever. Between the 5th and the 7th days, we performed IgM and IgG anti-dengue serologic examinations to confirm diagnosis of DHF. During hospitalization, the subject's clinical manifestations were observed, especially bleeding manifestations as a clinical outcome. Bleeding was classified as spontaneous bleeding on the days of observations such as ecchymoses, purpura, epistaxis, gum bleeding, hematemesis, melena, and hematuria.

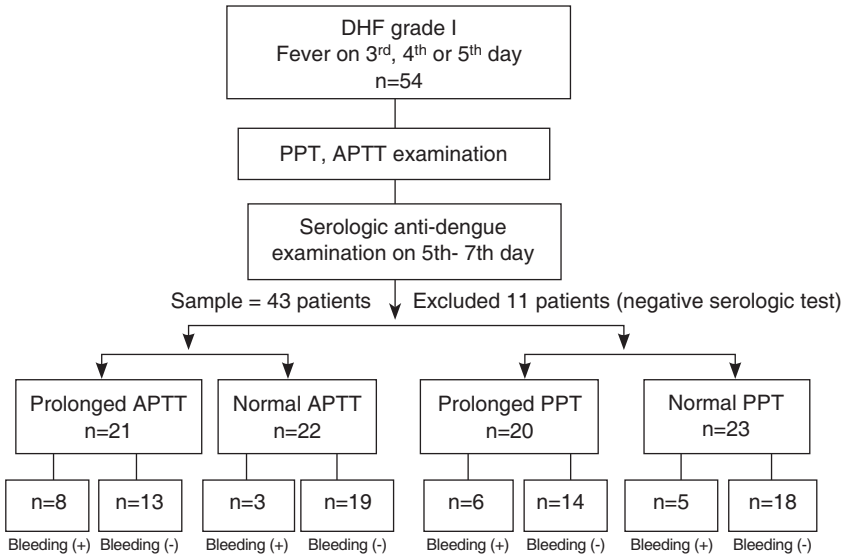


**Figure 1**. Scheme showing study design and results of APTT and PPT study results

Descriptive data are presented in narration and in tables. Primary outcomes (presence or absence of bleeding manifestations) were analyzed with the Kaplan-Meier method using median rates of PPT or APTT, and the day when bleeding manifestations appeared. Cox regression analysis was performed to adjust confounding variables that influence the outcome. Significance levels were $P < 0.05$ and 95% significance level.

## Results

During the study period, we recruited 131 patients with clinical diagnosis of DHF (66 cases of grade I DHF, 37 cases of grade II DHF, 21 cases of grade III DHF, and 7 cases of grade IV DHF). Only 54 patients with grade 1 DHF could be studied.

We first determined the cut-off point for APTT and PPT by calculating the median scores for these two parameters so that we could compare these with incidence of bleeding manifestations. We determined that the median PPT value was 12.8 seconds and median APTT value was 42 seconds. Based on these median rates as cut-off points, we assigned a normal PPT value as ≤12.9 seconds with a prolonged PPT value as >12.8 seconds, and a normal APTT value as ≤42 seconds with a prolonged APTT value as >42 seconds. Scheme of the study design with the results of the APTT and PPT tests in patients with grade 1 DHF is shown in **Figure 1**.

After examination of all the grade 1 DHF cases observed at the hospital, we found that, by the end of the study, 32 patients (74%) were without a bleeding manifestation and 11 patients (26%) showed a bleeding manifestation. Looking at the group with bleeding manifestations, we found eight patients (19%) with ecchymoses, purpura, gum bleeding, or epistaxis, and three cases (7%) with gastrointestinal bleeding (hematemesis or melena). The demographic characteristics of study subjects and the results of laboratory tests in relation to the APTT and PPT results are shown in **Table 1**.

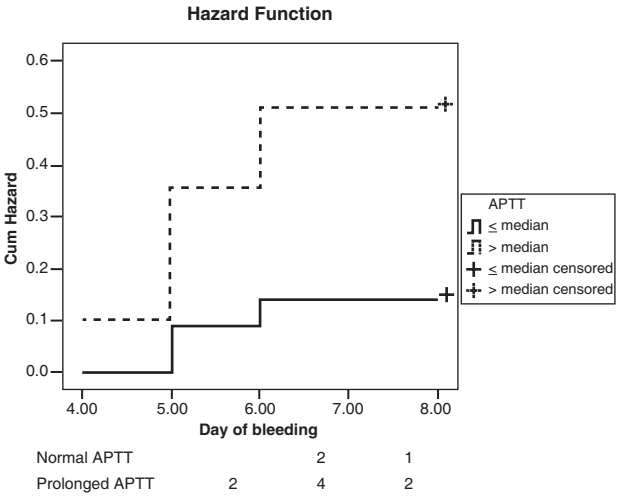


**Figure 2.** Hazard curve of bleeding manifestations in relation to APTT during observed period

**Table 1.** Characteristics of study subjects in relation to APTT and PPT results

| Characteristics | Prolonged APTT (n=21) | Normal APTT (n=22) | Prolonged PPT (n=20) | Normal PPT (n=23) |
|---|---|---|---|---|
| Age mean (SD) yr | 7.60 (2.66) | 7.47 (2.9) | 7.70 (2.64) | 7.39 (2.89) |
| Sex, n (%) | | | | |
| male | 11 (26) | 6 (14) | 8 (19) | 9 (21) |
| Nutritional status, n (%) | | | | |
| malnourished | 3 (7) | 3 (7) | 2 (4.7) | 4 (9) |
| normal | 18 (42) | 18 (42) | 18 (42) | 18 (42) |
| obese | 0 | 1 (2) | 0 | 1 (2) |
| Duration of fever at home, mean (SD) yr | 4.10 (0.7) | 4.09 (0.68) | 4.15 (0.59) | 4.04 (0.77) |
| Temperature, mean (SD) oC | 38.05 (1.02) | 37.82 (0.81) | 37.75 (1) | 38.09 (0.82) |
| Laboratory tests, mean (SD) | | | | |
| Lowest WBC, K/µL | 2.82 (1.19) | 3.26 (1.71) | 3.09 (1.35) | 3.01 (1.61) |
| Lowest HB, g/dL | 11.23 (0.99) | 11.14 (1.1) | 11.37 (1.12) | 11.02 (0.95) |
| Highest HCT, % | 46.81 (5.11) | 45.38 (5.27) | 47.74 (4.9) | 44.64(5.09) |
| Lowest PLT, K/µL | 30.58 (18.24) | 40.28 (23.42) | 28.99(17.52) | 41.24(23.1) |
| Bleeding manifestations, n (%) | 8 (18.6) | 3 (7) | 6 (11.6) | 5 (11.6) |

**Table 2.** Cox regression analysis of variables that influence to bleeding manifestations during DHF

| | B | P | Exp(B) | 95%CI |
|---|---|---|---|---|
| Age | 0.015 | 0.914 | 1.015 | 0.78 to 1.33 |
| Sex | -0.410 | 0.584 | 0.664 | 0.153 to 2.87 |
| Duration of fever | -0.398 | 0.467 | 0.672 | 0.23 to1.96 |
| Lowest PLT level | 0.002 | 0.911 | 0.998 | 0.96 to 1.04 |
| Highest HCT level | -0.025 | 0.725 | 0.975 | 0.85 to1.12 |
| APTT | 1.399 | 0.021 | 2.052 | 1.92 to 3.90 |
| PPT | -0.120 | 0.871 | 0.887 | 0.21 to 3.74 |

Using Kaplan-Meier analysis, we found that DHF patients who had a prolonged APTT value (greater than the median) showed significantly greater risk of bleeding compared to patients with a normal APTT value (less than or equal to the median) [RR 2.79 (95%CI 1.68 to 4.69), P <0.01], as presented in **Figure 2**.

Cox regression analysis was performed to adjust confounding variables such duration of fever at home, sex, age, lowest platelet level, highest hematocrit level, and PPT value. We found that there was no significance influence from those variables on DHF bleeding manifestations. We found that the only significance result was APTT value [RR 2.05 (95%CI 1.92 to 3.90), P =0.02], as seen on **Table 2**.

## Discussion

The main pathophysiologies of DHF that complicates the disease are plasma leakage due to increased capillary permeability, and hemostasis impairment. Shock and bleeding still remain the most dangerous clinical complications of DHF.

Bleeding manifestations in DHF are highly variable and ranging from a positive tourniquette test, mild bleeding from the skin such as petechie, ecchymoses, purpura, or hematoma at the site of injection, gum bleeding, epistaxis, to severe bleeding such as gastrointestinal tract bleeding, internal organ bleeding, and fatal intracranial bleeding. Gastrointestinal tract bleeding (hematemesis and/or melena) is the most frequent severe bleeding that leads to mortality. This study shows that all of the patients had a positive tourniquet test, eight patients (18.6%) with mild bleeding manifestations such epistaxis and gum bleeding, and three patients (7%) with severe bleeding such gastrointestinal tract bleeding or internal organ bleeding. There were nine patients (21%) had DSS with its complication of shock. No subjects died of their illness during this study.

A study by Phuong *et al*[12] who performed a prospective study of DHF in Vietnam in 1998 with total 319 DHF cases showed similar results to this study. In that study, 11% of cases showed spontaneous bleeding, and 12% of cases showed DSS and complications of shock while hospitalized. In 2001, Narayanan *et al*[13] studied clinical symptoms and laboratory tests used for monitoring DHF in relation to its complications found that 22% from total of 59 subjects who had a positive dengue serologic result also suffered from shock. In that study, 66.1% had bleeding manifestations. Based on a study conducted between February 2001 and April 2003 at Dr. Kariadi Hospital, Semarang, Indonesia, Setiati *et al*[14] found that the case fatality rate for DHF patients was 4%. Kalayanaroj *et al*[15] reviewed all data from DHF patients who were hospitalized in Bangkok Pediatric Hospital, Thailand, between 1995 and 1999, and found that 0.7% of cases were fatal.

The mechanism of bleeding during DHF still remains poorly understood. Hemostasis defects are caused by multiple factors such as vasculopathy (capillary and vein), thrombocytopenia, platelet dysfunction, and defects in the coagulation system (coagulopathy).[6-8] Several studies on the bleeding mechanism during DHF have identified that there was consumptive coagulation on most cases.[16] Hemostasis is maintained by the balance between the coagulation and fibrinolysis systems. During DHF, there is imbalance between these systems. Abnormal fibrinolysis can occur as a consequence of imbalances in the production of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1). Thrombocytopenia and plasma leakage during DHF cause a decrease in fibrinogen and coagulation factors (factor II, V, VII, IX, X, and XII). Defects in both systems will appear as bleeding, intravascular thrombosis or both. Most DHF cases with persistent shock are associated with coagulopathy. Persistent shock is often accompanied with metabolic acidosis and hypoxemia which leads to disseminated intravascular coagulation (DIC) that worsens the bleeding.[8,17,18]

PPT and APTT are indicators of the extrinsic and intrinsic coagulation system during the cascade. This study found that there was a significant relationship between prolonged APTT during the early stages of DHF with bleeding manifestations at the later stage of the disease [RR: 2.79 (95% CI 1.68 to 4.69), $P < 0.01$]. From cox regression analysis, it was found that only APTT appeared to be correlated with bleeding manifestations [RR 2.05 (95%CI 1.92 to 3.90), $P = 0.02$]. This result shows that APTT is a predictor of bleeding manifestations during DHF.

A prolonged APTT value during DHF indicates that there is a defect in the intrinsic cascade of coagulation system. During the acute phase of dengue viral infection, there is an increase in capillary permeability due to release of inflammatory mediators such as cytokine, C3a, C5a, TNF-α, interleukin (IL)-2, IL-6, IL-10, interferon-α, and histamine. Increase of capillary permeability leads to plasma leakage, which causes the decrease of fibrinogen and coagulation factors (factor II, V, VII, VIII, IX, X, and XII), also increase of consumption. In addition, liver cells can be damaged by inflammation; this can be seen by an increase in aspartat aminotransferase (AST) or alanine aminotransferase (ALT) levels. Damage to the liver cells further decreases coagulation factors synthesis. APTT has a strong relationship with both AST/ALT increases and decreases in coagulation factors due to low synthesis or high consumption; this will prolong APTT value during the acute stage of DHF.[8,17,18] If DHF is accompanied by severe bleeding complications and there is possibility of DIC, this indicates that there are defects in the intrinsic and extrinsic cascades.

Another study that showed had similar results was that of Krishnamurti *et al*[19] who conducted a prospective study on the bleeding mechanism during DHF without shock. The study found that prolonged APTT had significant relationship with the mean rate of APTT. In addition, a study by Liu *et al*[20] in Taiwan also showed similar results; a total of 450 cases during an epidemic were examined and prolonged APTT was found in more than 20% cases.

Wills *et al*[11] studied coagulation defects during DHF in 167 children diagnosed with DSS in Vietnam between July 1998 and February 1999. The study found prolonged APTT and PPT in DSS patients with bleeding. This different result is because all of the subjects had DSS with DIC complication; in DIC cases there is always prolonged APTT and PPT.

In conclusion, our data indicate that APTT values may be used as a predictor for bleeding manifestations in DHF. Further studies are needed to confirm our findings in different patient populations.

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## References

1. World Health Organization. Dengue hemorrhagic fever diagnosis, treatment, prevention and control. 2nd ed. Geneva: WHO; 1997.
2. Halstead SB. Dengue fever/dengue hemorrhagic fever. In: Behrman RE, Kliegman RM, Jenson HB, editors. Nelson Textbook of Pediatrics. 16th ed. Philadelphia: WB Saunders, 2000; p. 1005-8.
3. Cherry JD. Dengue and dengue hemorrhagic fever. In: Feigin RD, Cherry JD, editors. Textbook of pediatric infectious diseases. Philadelphia: WB Saunders,1991; p. 1140-8.
4. Djajadiman Gatot. Perubahan hematologi pada infeksi dengue. Presented at Pelatihan bagi Pelatih Dokter Spesialis Anak dan Dokter Spesialis Penyakit Dalam dalam Tatalaksana kasus DBD, Jakarta, November 24-26, 1998.
5. Sutaryo, Pudjo Hagung Wijayanto. Patogenesis koagulopati pada demam berdarah dengue. In: Sutaryo, Pudjo Hagung W, Sri Mulatsih, editors. Tatalaksana syok dan perdarahan pada DBD. Yogyakarta: Medika FK UGM, 2004; p. 30-9.
6. Guzman MG, Kouri G. Dengue: an update. Lancet. 2002;2:33-42.
7. van Gorp ECM, Suharti C, ten Cate H, Dolmans WMV. Review: Infectious Diseases and Coagulation Disorders. J Infect Dis. 1999;180:176-86.
8. Nimmannitya S. Dengue Hemorrhagic Fever: Disorders of hemostasis. Bangkok J Pediatrics.1999; 24:184-7.
9. McBride W, Ohmann HB. Dengue viral infections: Pathogenesis and Epidemiology. Microbes and Infection. 2000;2:1041-50.
10. Guzman MG, Kouri G. Advances in dengue diagnosis. Journal of Clinical and Diagnostic Laboratory Immunology. 1996;3:621-7.

11. Wills BA, Oragui E E, Stephens A C, Daramola OA, Dung N M, Loan HT, *et al*. Coagulation Abnormalities in Dengue Hemorrhagic Fever: Serial Investigations in 167 Vietnamese Children with Dengue Shock Syndrome. J Clin Infect Dis. 2002;35:277-85.
12. Phuong CXT, Nhan NT, Thuy PTT, Thien CV, Nga NTT, Thuy TT *et al*. Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? Am J Trop Med Hyg. 2004;70(2):172-9.
13. Narayanan M, Aravind MA, Ambikapathy P, Prema R, Jeyapaul MP. Dengue fever-clinical and laboratory parameters associated with complications. Dengue Bulletin. 2003;27:108-15.
14. Setiati TE, Mairuhu ATA, Koraka P, Supriatna M, Gillavry MRM, Brandjes DPM *et al*. Dengue disease severity in Indonesian children: an evaluation of the World Health Organization classification system. BMC Infectious Diseases. 2007;7(22):1-8.
15. Kalayanarooj S, Chansiriwongs V, Nimmannitya S. Dengue patients at the Children's Hospital, Bangkok: 1995-1999 review. Dengue Bulletin. 2002;26:33-43.
16. Mitrakul C. Bleeding problems in dengue hemorrhagic fever: platelet and coagulation changes. Southeast Asian J Trop Med Pub Hlth 1987; 18:407-12.
17. Lei H Y, Yeh T M, Liu H S, Lin Y S. Immunopathogenesis of Dengue Virus Infection. J Biomed Sci. 2001;8:377-88.
18. Chuansumrit A, Tangnararatchakit K. Pathophysiology and management of dengue hemorrhagic fever. Journal Compilation Transfusion Alternatives in Transfusion Medicine. 2006;8:3-11.
19. Krishnamurti C, Kalayanarooj S, Cutting MA, Peat RA, Rothwell AW, Reid TJ, *et al*. Mechanisms of hemorrhage in dengue without circulatory collapse. Am J Trop med Hyg. 2001;65(6):840-7.
20. Liu JW, Khor BS, Lee CH, Lee IK,Chen RF, Yang KD. Dengue hemorrhagic fever in Taiwan. Dengue Bulletin. 2003;27:19-24.