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Bleeding outcome during a dengue outbreak in 2005 in the East-coast region of Peninsular Malaysia: a prospective study

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Abstract. During a dengue outbreak in 2005 in the East-coast region of Peninsular Malaysia, one of the worst hit areas in the country at that time, we undertook a prospective study. We aimed to describe the bleeding outcome and changes in the liver and hematologic profiles that were associated with major bleeding outcome during the outbreak. All suspected cases of dengue admitted into the only referral hospital in the region during the outbreak were screened for WHO 2002 criteria and serology. Liver function, hematologic profile and severity of bleeding outcome were carefully documented. The association between symptoms, liver and hematologic impairments with the type of dengue infection (classical vs. hemorrhagic) and bleeding outcome (major vs. non-major) was tested. Dengue fever was confirmed in 183 cases (12.5/100,000 population) and 144 cases were analysed. 59.7% were dengue hemorrhagic fever, 3.5% were dengue shock syndrome and there were 3 in-hospital deaths. Major bleeding outcome (gastrointestinal bleeding, intracranial bleeding or haemoptysis) was present in 14.6%. Elevated AST, ALT and bilirubin were associated with increasing severity of bleeding outcome (all $P < 0.05$). Platelet count and albumin level were inversely associated with increasing severity of bleeding outcome (both $P < 0.001$). With multivariable analysis, dengue hemorrhagic fever was more likely in the presence of abdominal pain (OR 1.1, 95% CI 0.02-1.6) and elevated AST (OR 1.0, 95% CI 1.0-1.1) but the presence of pleural effusion (OR 5.8, 95% CI: 1.1-29.9) and elevated AST (OR 1.008, 95% CI: 1.005-1.01) predicted a severe bleeding outcome. As a conclusion, the common presence of a severe hemorrhagic form of dengue fever may explain the rising death toll in recent outbreaks and the worst impairment in liver and hematologic profiles was seen in major bleeding outcome.

INTRODUCTION

Dengue outbreak carries a serious morbidity and mortality in many tropical countries including Malaysia (Kurane, 2007; Guzman & Isturiz, 2010;). More than half million cases of dengue hemorrhagic fever are being reported to World Health Organization (WHO) annually (Kurane, 2007). Mortality rates were reported be between 1-5% in those patients presenting with dengue shock syndrome (Kurane, 2007). Recent data indicates a rising death toll resulting from frequent outbreaks of dengue within the South-East Asia including Malaysia. The total reported cases of dengue fever in Malaysia were 49, 355 patients with 138 deaths between the year 2004 to 2005 (Shepard, 2013).

With a rising incidence of dengue fever within the country, especially complicated dengue, there is a desperate need to identify markers of severity to allow for early medical interventions. Both liver and hematologic impairments are signs of possible complicated course of dengue and are potential targets for severity assessment (Nguyen *et al.*, 1997; Huerre *et al.*, 2001; Souza *et al.*, 2004; Martina *et al.*, 2009). Liver impairment

in complicated dengue tends to occur early and can be severe according to previous studies. Nimmannitya *et al.* (1987) reported elevated alanine transaminase (ALT) in 25% of patients with dengue fever. A study from Brazil similarly observed an elevation of aspartate transaminase (AST) level in 63.4% and ALT in 45% of patients with dengue fever (Souza *et al.*, 2004). The elevation of liver enzymes is usually mild to moderate in severity but acute hepatitis with more than 10-fold rise was reported in 3.8% of cases (Souza *et al.*, 2004).

It is still unclear why, in dengue fever, AST elevation predominates in certain populations and ALT elevation predominates in others. A study from Thailand showed that AST was more significantly elevated (Wichmann *et al.*, 2004), in contrast, another study from Taiwan showed that ALT was more significantly elevated (Lee *et al.*, 2006). A previous retrospective study in Malaysia involving 50 patients had instead showed a higher ALT and alkaline phosphatase (ALP) levels in dengue hemorrhagic fever but the association was not statistically significant (Wahid *et al.*, 2000).

During a dengue outbreak in 2005 in the East-coast region of Peninsular Malaysia, one of the worst hit areas in the country at that time, we undertook a prospective study. We aimed to describe the bleeding outcome and changes in the liver and hematologic profiles that were associated with complicated dengue especially those patients having a major bleeding outcome during the outbreak.

MATERIALS AND METHODS

Subjects

This was a prospective cross-sectional study based at Hospital Tengku Ampuan Afzan (HTAA), the only tertiary hospital in the whole state of Pahang. Pahang is the largest state in Malaysia and is situated in the more rural and less developed region of Peninsular Malaysia with a population of approximately 1.2 million. This study took place during a dengue outbreak occurring between the months of July 2005 and June 2006. The study was approved by the Medical Advisory and Ethical committee of HTAA.

Patients with clinically WHO 2002 criteria (Gautret *et al.*, 2012) and serologically confirmed dengue infection during the outbreak and who were admitted into the medical wards of the hospital were included into the study. Exclusion criteria included patients having concomitant chronic liver disease or liver malignancy, acute and chronic hepatitis not secondary to dengue, paracetamol overdose, alcohol, the presence of other infections being associated with liver impairment (including typhoid, malaria, melioidosis and leptospirosis) and patients with a known bleeding disorder.

All patients recruited into the study would have their demographic data, symptoms, bleeding outcome, physical findings, complications and blood investigation results being captured in careful details. Blood investigations that were carried out during the hospital admission have included the following: full blood counts, hematocrit, prothrombin time (PT), activated prothrombin time (aPTT), International Normalized Ratio (INR), albumin, direct bilirubin, alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP). Rapid serological tests, 'Panbio' Dengue Duo IgM and IgG Rapid Strip Test (category no: R-DEN02D), were used for initial detection of dengue infection (Lam *et al.*, 1998). Serological confirmation of dengue infection was performed with 'Panbio' Dengue IgM Capture enzyme-linked immunoabsorbent assay (ELISA) (category no: E-DEN01M) and "Panbio' Dengue IgG Capture ELISA (category no: E-DEN02G) (Lam *et al.*, 1998). These serological tests had been previously validated for use in the local population (Lam *et al.*, 1998; Kumarasamy *et al.*, 2007).

The study was approved by the Medical Advisory and Ethical committee of HTAA. Subjects were recruited into the study after providing written informed consent.

Definitions

Dengue fever (DF) was diagnosed based on the WHO 2002 criteria which include

a clinical criteria of acute febrile illness and have two or more of the following manifestations: headache, retro orbital pain, myalgia, athralgia, rash, haemorrhagic manifestations or leucopenia, and a positive serological criteria (Gautret *et al.*, 2012). A positive serology was defined as the presence of positive IgM antibodies at 5 or more days from the onset of fever with or without IgG antibodies by either Rapid Strip Test or ELISA. Equivocal results were taken as negative result. A positive IgG antibody alone was not considered positive for the serology criteria because IgG antibodies could be persistent for more than 10 months and prone to false positives partly due to their cross-reaction with other flavivirus antigens. Primary infection was defined as positive IgM antibodies but negative for IgG antibodies. Secondary infection was defined as positive for both IgM and IgG antibodies.

Dengue hemorrhagic fever (DHF), the complicated form of dengue fever, was defined according to the WHO 2002 criteria as the presence of fever (or history of fever), plasma leakage, thrombocytopenia with platelet count < $100x10^3/mm^3$ and hemorrhagic tendencies in patients with confirmed diagnosis of dengue infection. Plasma leakage was defined as either greater than 20% rise in hematocrit level appropriate for age and gender or greater than 20% drop in hematocrit following volume replacement compared with baseline and or supported by clinical signs including pleural effusion, ascites and hypoalbuminemia.

Bleeding outcome of dengue infection was determined by the most serious site of bleeding occurring during admission as witnessed by a single investigator (FS) who was blinded to the study. The scoring was adapted from a study by Krishnamurti *et al.* (2001) which include the following: 1 – No bleeding, 2 – Petechiae, 3 – Minor bleeding (epistaxis or gingival bleeding) and 4 - Major bleeding (gastrointestinal bleeding, intracranial bleeding or haemoptysis).

Data and statistical analysis

Analysis was performed using SPSS software version 12.0.1. Categorical variables were recorded as frequencies and percentages and numerical variables were recorded as means and standard deviations (SD) unless otherwise stated. Univariate analysis on categorical variables was analysed using Chi-Square or Fischer-Exact test. Numerical variables with at least 2 factors were analysed using independent t-test but if there were more than 2 factors, then Kruskal-Wallis test and Mann-Whitney test (with Bonferroni adjustment for *P*-value) were used. Variables from hematologic and liver profiles with statistical significance below 0.05 from univariable analysis were subsequently tested for their association with major vs. without major bleeding outcome. Receiver operating characteristics (ROC) curve analysis was used to determine the area under the curve (AUC) for liver enzymes (AST, ALT and ALP) as markers for dengue hemorrhagic fever and major bleeding outcome. Variables significant from univariable analyses were also tested using multiple logistic regression analysis. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Population characteristics of dengue outbreak

There was a total of 386 suspected cases (26.3 cases/100,000 population) of dengue during the year of outbreak and 183 cases (12.5 cases/100,000 population) were later confirmed to have dengue fever, based upon the WHO 2002 criteria (Gautret *et al.*, 2012). Of the 183 patients, 144 were included into the final analysis with 39 patients being excluded for the following reasons: 26 having poor or incomplete blood tests, 6 had HIV and or Hepatitis C infection, 5 had Hepatitis B infection and 2 had Melioidosis infection.

Based on the WHO criteria, 40.3% (58/144) had classical or uncomplicated dengue fever and 59.7% (86/144) with DHF, of which 3.5% (5/144) met the criteria for dengue shock syndrome (or DSS). Of the 5 subjects with DSS, 3 eventually died. The mean age of 144 patients was 30.9 ± 12.2 years, with 56.3% (81/144) being males and 43.8% (63/144) being females. Ethnic Malays were the majority with 67.4% of total cases (97/144)

followed by the Chinese with 12.5% of total cases (18/144) and Indians with 9% of total cases (13/144). There was no difference in distribution between age, gender and ethnicities with classic or dengue hemorrhagic fever (Table 1).

Clinical characteristics of dengue outbreak

Compared to classical dengue, abdominal pain (37.2% vs. 17.2%, $P = 0.01$) and hepatomegaly (53.5% vs. 22.4%, $P < 0.001$) were more common in DHF (Table 1). Likewise, plasma leakage (93.0% vs. 17.2%, $P = 0.02$) and its clinical sign, pleural effusion (12.8% vs. 1.7%, $P < 0.001$) but not ascites, were more common in DHF than classical dengue (Table 1).

Minor bleeding was the most common bleeding outcome, being present in 36.8% (53/144) of patients, followed by petechiae (27.1% or 39/144) and major bleeding was seen in 14.6% (21/144) (Figure 1). Primary infection was equally diagnosed in both classical and DHF but secondary infection was more common in DHF (55.8% vs. 37.9%, $P = 0.03$).

Hematologic impairment

There was no significant difference in distribution between white cell count, hemoglobin and hematocrit on admission

Table 1. Characteristics of dengue fever outbreak

Parameters	Type of Dengue fever			Major bleeding		
	Classic (n = 58)	DHF (n = 86)	*P* value	Not major (n=123)	Major (n = 21)	*P* value
Age, years, mean (SD)	29.6 (12.5)	31.8 (12.1)	0.3	30.9 (12.9)	30.4 (10.2)	0.9
Gender, n (%)						
Male	36 (62.1)	45 (52.9)	0.2	72 (58.5)	9 (42.9)	0.1
Female	22 (37.9)	41 (48.2)		51 (41.5)	12 (57.1)	
Ethnics, n (%)						
Malays	37 (63.8)	60 (69.8)	0.5	81 (65.9)	16 (76.2)	0.4
Non-Malays	21 (36.2)	26 (30.2)		42 (34.1)	5 (23.8)	
Clinical features						
Abdominal pain	10 (17.2)	32 (37.2)	0.01#	30 (24.4)	12 (57.1)	0.004#
Ascites	0 (0)	3 (3.5)	0.1	1 (0.8)	2 (9.5)	0.06
Pleural effusion	1 (1.7)	11 (12.8)	< 0.001#	6 (4.9)	6 (28.6)	0.002#
Plasma leakage	10 (17.2)	80 (93.0)	0.02#	72 (58.5)	19 (90.5)	0.006#
Hepatomegaly	13 (22.4)	46 (53.5)	< 0.001#	44 (35.8)	15 (71.4)	< 0.001#
Serological classification						
Primary (IgM only)	36 (62.1)	38 (44.2)	0.5	45 (36.6)	10 (47.6)	0.6
Secondary (IgM and IgG)	22 (37.9)	48 (55.8)	0.03#	21 (17.1)	5 (23.8)	0.3
Hematologic profile, mean (SD)						
White cell count, $x10^9$/L	3.9 (2.0)	3.4 (1.9)	0.5	3.5 (1.8)	4.3 (2.9)	0.09
Hemoglobin, g/dl	13.9 (1.8)	14.4 (2.2)	0.2	13.5 (1.6)	15.9 (3.2)	0.1
Hematocrit, %	40.4 (4.9)	41.5 (5.9)	0.2	41.1 (6.5)	40.9 (6.5)	0.9
Platelet, $x10^9$/L	116.6 (71.2)	50.3 (36.1)	< 0.001#	53.0 (34.9)	81.1 (64.9)	0.05
aPTT, s	32.1 (9.1)	35.4 (9.1)	0.01#	36.4 (11.0)	33.6 (7.4)	0.1
INR, ratio	1.0 (0.1)	1.1 (0.4)	0.5	1.0 (0.4)	1.1 (0.2)	0.6
Liver function test, mean (SD)						
Albumin, g/L	38.0 (4.4)	32.0 (5.7)	< 0.001#	35.1 (5.7)	30.3 (5.1)	< 0.001#
Direct Bilirubin, µmol/L	3.9 (1.9)	6.2 (5.4)	< 0.001#	4.9 (3.8)	7.7 (6.9)	0.01#
ALT, IU/L	42.8 (31.3)	171.1 (185.2)	< 0.001#	88.0 (86.7)	303.3 (299.7)	< 0.001#
AST, IU/L	64.7 (46.5)	253.5 (205.5)	< 0.001#	133.1 (119.9)	437.3 (276.8)	< 0.001#
ALP, IU/L	82.5 (32.1)	97.6 (48.9)	0.03#	89.8 (42.8)	101.7 (47.0)	0.2

Significant $P < 0.05$

SD; standard deviation, aPTT; activated prothrombin time, INR; internationalized normal ratio

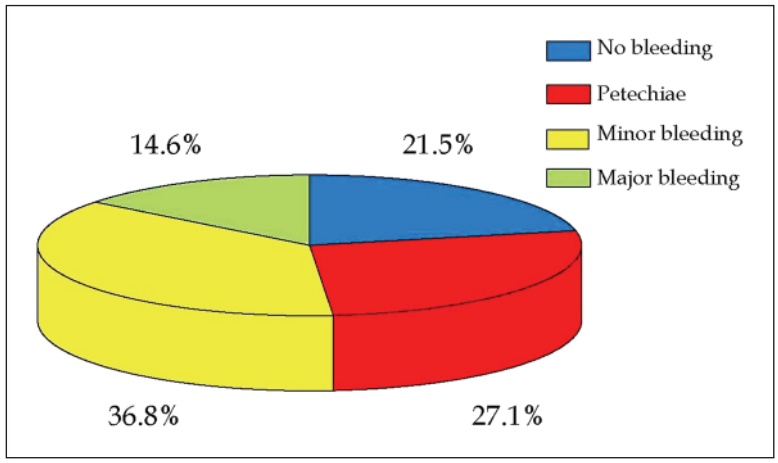

Figure 1. Bleeding outcome in patients admitted with dengue infection

with classical or DHF (Table 1). While INR was not significantly different between classical and DHF, aPTT was only slightly more prolonged in DHF than classical fever (35.4 ± 9.1 s vs. 32.1 ± 9.1 s, $P = 0.01$).

Platelet count was significantly lower with hemorrhagic fever as compared to classical fever (50.3 ± 36.1 x10^9/L vs. 116.6 ± 71.2 x10^9/L, $P < 0.001$). In those patients with plasma leakage compared to those without, platelet count was also lower, being 53.0 ± 39.7 x10^9/L and 118.9 ± 71.1 x10^9/L respectively, $P < 0.001$. Likewise, platelet count, compared to no bleeding (124 ± 50 x10^9/L), was significantly lower, after Bonferroni adjustment, in those patients with petechiae (69.0 ± 62.0 x10^9/L, $P < 0.001$), with minor bleeding (40.0 ± 50.0 x10^9/L, $P < 0.001$) and with major bleeding (44.0 ± 12.5 x10^9/L, $P < 0.001$). No association was seen between aPTT and different severity of bleeding outcome.

Liver impairment

As expected, the albumin level was significantly lower in DHF as compared to classical fever (32.0 ± 5.7 vs. 38.0 ± 4.4 g/L, $P < 0.001$) but total bilirubin was higher in DHF than classical fever (6.2 ± 5.4 vs. 3.9 ± 1.9 µmol/L, $P < 0.001$) (Table 1). Similarly, albumin level was inversely associated with increasing bleeding severity ($P < 0.001$) and total bilirubin was greater with increasing bleeding severity ($P = 0.04$). Albumin level was lower in the presence vs. absence of plasma leakage (27.8 ± 5.3 vs. 31.2 ± 3.6 g/L, $P < 0.001$) and pleural effusion (28.5 ± 8.1 vs. 34.9 ± 5.3 g/L, $P < 0.001$).

Liver enzymes were more elevated in DHF vs. classical fever, with AST (253.5 ± 205.5 IU/L vs. 64.7 ± 46.5 IU/L, $P < 0.001$) being observed to have a greater rise, followed by ALT (171.1 ± 185.2 IU/L vs. 42.8± 31.3 IU/L, $P < 0.001$) and a lower rise was seen with ALP (97.6 ± 48.9 IU/L vs. 82.5 ± 32.1 IU/L, $P = 0.03$) (Table 1). Both AST ($P < 0.001$) and ALT ($P < 0.001$) were also found to be elevated with severe bleeding outcomes but not ALP (Table 2).

ROC curve analysis was used to determine the AUC for the three liver enzymes; AST, ALT and ALP with regards to their reliability in predicting presence of DHF and major bleeding outcome (Figure 2). For DHF, the AUCs for AST, ALT and ALP were 0.91 (95% CI 0.86 – 0.96, $P < 0.001$), 0.87 (95% CI: 0.82 – 0.93, $P < 0.001$) and 0.58 (95% CI: 0.49 – 0.67, $P = 0.1$) respectively. For major bleeding outcome, the AUCs for AST, ALT and ALP were 0.91 (95% CI: 0.85 – 0.96, $P < 0.001$), 0.86 (95% CI: 0.78 – 0.95, $P < 0.001$) and 0.58 (95% CI: 0.44 – 0.72, $P = 0.3$) respectively.

Multivariable analysis

Dengue hemorrhagic fever was associated with abdominal pain (odds ratio, OR 1.1, 95% CI: 0.02 – 1.6) and elevated AST level (OR 1.0, 95% CI: 1.0 – 1.1) (Table 3). On the other

Table 2. Association between blood and liver profile and different severity of bleeding outcome

Parameters	No bleed	Petechiae	Minor bleed	Major bleed
Blood profile				
Platelet, x10^9/L	124.0 (50.0)	69.0 (62.0)	40.0 (50.0)	44.0 (125.0)
aPTT, s	30.3 (5.4)	34.1 (8.4)	35.3 (7.1)	36.4 (11.0)
Liver profile				
Albumin, g/L	39.2 (2.8)	35.0 (5.8)	33.6 (6.2)	30.0 (7.0)
Bilirubin, µmol/L	3.2 (1.6)	3.0 (5.1)	4.2 (3.4)	5.2 (7.4)
ALT, IU/L	27.0 (22.0)	66.0 (61.0)	89.0 (86.0)	183.0 (189.0)
AST, IU/L	34.0 (22.0)	96.0 (103.0)	146.0 (107.0)	403.0 (323.0)
ALP, IU/L	78.0 (31.0)	80.0 (44.0)	84.0 (37.0)	88.0 (63.0)

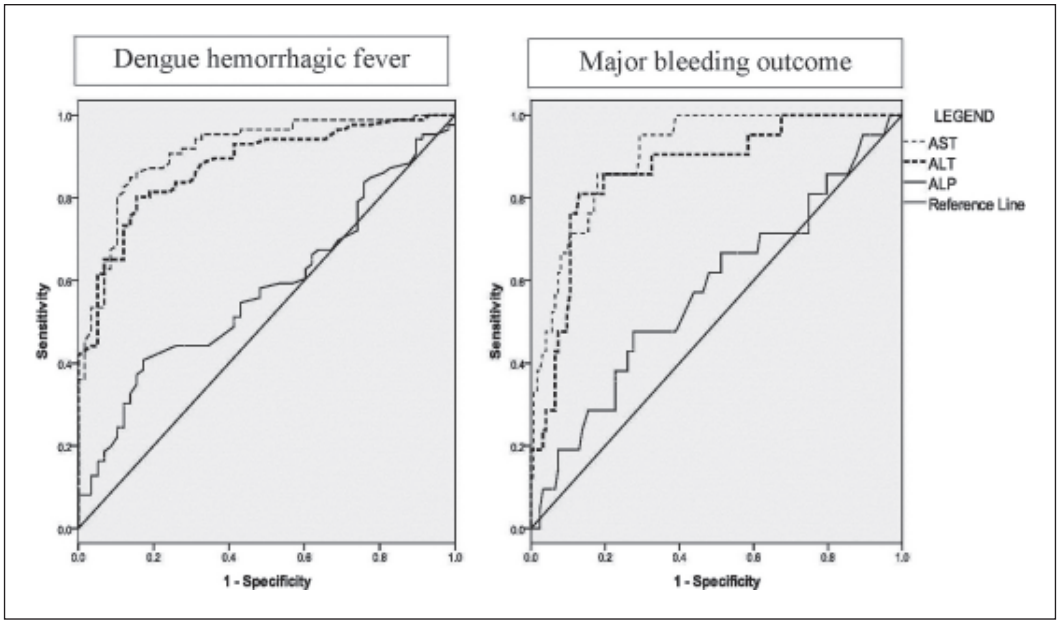

Figure 2. Receiver operating characteristics (ROC) curve analysis on the reliability of liver enzymes (AST, ALT and ALP) to determine the risk of dengue hemorrhagic fever and major bleeding outcome

Table 3. Multivariable analysis of parameters associated with complicated dengue and major bleeding outcome

Parameters	OR	95% CI for OR	*P* value
Hemorrhagic dengue fever			
Abdominal pain	1.1	0.01 – 1.6	0.01
AST (IU/L)	1.0	1.01 – 1.05	0.001
Major bleeding outcome			
Pleural effusion	5.8	1.1 – 29.9	0.03
AST (IU/L)	1.008	1.005 – 1.01	< 0.001

OR; adjusted odd ratio, CI; confidence interval

hand, major bleeding outcome in dengue was associated with elevated AST level (OR 1.008, 95% CI 1.005 – 1.01) and in the presence of pleural effusion (OR 5.8, 95% CI: 1.1 – 29.9).

DISCUSSION

Our prospective study in a rural-majority population in the East-coast region of Peninsular Malaysia, one of the worst hit areas of dengue outbreak in the year of 2005, had found a predominance of DHF of almost 60%, and this was similar to a study in Chonburi, at the eastern-coast of the Gulf of Thailand, which had reported a rate of 63% (Wichmann *et al.*, 2004). In contrast, DHF was reported in only 9% of admitted cases in Brazil (Souza *et al.*, 2004) and 36% of confirmed dengue in Taiwan (Lee *et al.*, 2006). The predominance of complicated dengue or DHF in our population explains the rising mortality seen in recent outbreaks. Furthermore, secondary rather than primary infection was found to be more common with DF in this population. It must be noted, however, a diagnosis of DF in our study was based on the WHO 2002 guideline rather than the newer published guideline in 2009 because of the study being conducted in 2005.

Patients affected with dengue were relatively young adults, with a mean age of 30.9 years but no difference in age was seen between classical dengue and DHF, and likewise with vs. without major bleeding outcome (Table 1). More developed countries and urban area may see a more complicated course of dengue in the elderly, since they often have other co-morbidities (Lee *et al.*, 2006; Thomas *et al.*, 2010; Gautret *et al.*, 2012). However, a similar age pattern to our population was also observed in the Kuala Lumpur city during the same time period (Sam *et al.*, 2013). This reflects a changing pattern of DF in Malaysia, from a predominant disease of children to a disease of young adults, and there is no longer a rural-urban divide (Jamaiah *et al.*, 2005; Muhammad *et al.*, 2011; Hassan *et al.*, 2012).

In contrast to other reported studies from Malaysia, ours did not suggest a gender difference between classical dengue and DHF despite a similar predominance of males (Table 1). Similarly, no difference was seen in gender with vs. without major bleeding outcome. Females were found to be associated with more case fatalities in those studies but retrospective designs, differences in ethnic distribution and the presence of co-morbidities might have biased their results (Sam *et al.*, 2013; Wallace *et al.*, 1980). Reported studies from other countries are also variable with respect to gender difference and DHF, with some having a female predilection (Pang *et al.*, 2012; Guerra-Silveira & Abad-Franch, 2013,) and another study with females being protected from DHF (Moraes *et al.*, 2013).

Clinical features including abdominal pain, hepatomegaly and pleural effusion were more commonly associated with DHF and major bleeding outcome in this population (Table 1). These three features were among the seven warning signs recently proposed by the WHO and Special Program for Research and Training in Tropical Diseases (TDR) in 2009 (WHO, 2009). Studies suggest that these signs were of practical value in predicting disease progression in a majority of younger patients. A recent report from Singapore indicates a less enthusiasm in this regard where no warning signs were found to be of high sensitivity even though they were highly specific (Thein *et al.*, 2013). Multivariable analysis in our study indicated that abdominal pain and pleural effusion were significant indicators of DHF and major bleeding outcome respectively (Table 3). While hepatomegaly and pleural effusion are related to plasma leakage, which is one of the WHO criterion for DHF, the exact origin of abdominal pain has remained obscure with some studies suggest this being due to acalculous cholecystitis, mild pancreatitis or hemorrhagic gastritis (Sharma *et al.*, 2006; Setiawan *et al.*, 1998; Wang *et al.*, 1990).

Minor bleeding in the form of mucosal bleeding was the most common bleeding outcome observed in our study (Figure 1). Mucosal bleeding is a useful warning sign for complicated dengue and this was proposed by WHO and TDR in 2009 (WHO, 2009). On the other hand, major bleeding was seen in 14.6% of our study population, a rather

frequent and severe outcome. Of the hematologic abnormalities, a lower platelet count (mean 50.3 x 10^9/L) and a more prolonged aPTT (mean 35.4 s) were associated with DHF but not hematocrit and INR. A lower platelet count was also significantly associated with plasma leakage and all spectrum of bleeding outcomes in DHF. The pathogenesis of thrombocytopenia is largely due to increased destruction of platelets as a result of complement activation, peripheral sequestration and marrow dysfunction (Krishnamurti *et al.*, 2001) and more recently, oxidative stress (Soundravally *et al.*, 2008). Platelets are also shown to preferentially adhere to dengue virus-stimulated endothelial cells (Krishnamurti *et al.*, 2002).

Liver impairment was common in DHF with a 10-fold elevation of AST and ALT being observed in 12.5% and 4.2% of patients respectively. Several reported studies have linked both elevations in AST and ALT as predictors for a more complicated course in dengue (Kuo *et al.*, 1992; Nguyen *et al.*, 1997). Our study further indicates that between the two, AST was more predictive of DHF and major bleeding outcome according to multivariable analysis (Table 3). The value of liver aminotransferases to discriminate the severity of DF has been questioned in a recent retrospective study from Singapore (Lee *et al.*, 2012). Despite being associated with DHF, the authors from Singapore claimed that the AUC for AST and ALT were 0.56 and 0.55 respectively, and both were insufficient to discriminate between uncomplicated and complicated dengue. Besides a retrospective design, this study suffered by not analysing AST and ALT with respect to different severity of bleeding outcomes, especially major bleeding outcome, which is often associated with a poorer prognosis. We found that increasing elevations in AST and ALT were associated with increasing severity of bleeding outcomes (Table 2). Elevation in AST appeared to be greater than elevation in ALT with severe bleeding outcomes and this was especially so in patients having a major bleed. Unlike AST and ALT, ALP did not show any association with severe bleeding outcomes despite being more elevated in DHF. In addition, from the ROC curve analysis, we were able to demonstrate AST and ALT being significant predictors of DHF and also major bleeding outcome but not ALP. This suggests that the pathogenesis of liver impairment in dengue hemorrhagic fever is largely hepatocellular rather than hepatobiliary (Parkash *et al.*, 2010). However, liver impairment alone does not fully explain the larger elevation of AST compared to the ALT. A most recent report suggests that the predominant source of AST was from the muscles rather than the liver, with the AST levels positively correlated with creatine kinase level, especially during the first 8 days (Tristao-Sa *et al.*, 2012).

A lower albumin but a higher bilirubin levels were observed in patients with DHF vs. classical dengue (Table 1). Likewise, a reduction in albumin but an increased bilirubin levels was associated with severe bleeding outcome (Table 2). Previous studies have reported an association between hypoalbuminemia and DHF but these patients often presented with a most severe form of dengue and had high mortality rates (Brito *et al.*, 2007; Ong *et al.*, 2007). Albumin level was shown to be inversely associated with the presence of plasma leakage and pleural effusion in the current study; this suggests that a low albumin level is a marker of vascular leakage rather than synthetic dysfunction of the liver. With acute hepatitis being common in complicated dengue, bilirubin was expected to increase in proportion with the severity of liver inflammation (Parkash *et al.*, 2010).

The current study has limitations. Not all patients during the outbreak were captured by HTAA but this is the only tertiary hospital within the state of Pahang. Some of the rural population might not have sought treatment, or were attended by traditional healers or were treated in smaller clinics and community hospitals without referring to HTAA. Mortality and morbidity after hospital discharge had not been assessed, but in-hospital mortality was not common as was shown in the current study, with three deaths from shock. However, the strength of the

current study was its prospective design and its data collection during an actual outbreak unlike other retrospective studies within the region.

As a conclusion, the common presence of a severe hemorrhagic form of dengue fever may explain the rising death toll in recent outbreaks and the worst impairment in liver and hematologic profiles was seen in major bleeding outcome. The findings of abdominal pain, pleural effusion and elevated AST during admission are warning signs for a possible impending severe bleeding outcome. Patients with these warning features will need early referral to a tertiary institution.

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