BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	Draft
Article Type:	Research
Date Submitted by the Author:	n/a
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	Dengue, Hospital stay, Mortality, Risk Factors, Dengue Viral Infection

Determinants of Mortality and Prolonged Hospital stay among Dengue patients attending Tertiary Care Hospital: A Retrospective Analysis

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Running title: Dengue related prolonged hospital stay and mortality

Keywords: Dengue; Hospital stay; Mortality; Risk Factors; Dengue Viral Infection; Dengue Hemorrhagic fever

Word count: 4238 words

Abstract

Abstract

Objectives: Dengue imposes substantial economic and disease burden to society and health care system in terms of hospital stay, morbidity and mortality. Early identification of dengue cases with high propensity of increased hospital stay and death could be of value in isolating patients in need of early interventions. Current study was aimed to determine the significant factors associated with dengue-related prolonged hospitalization and death.

Design: Cross-sectional retrospective study

Setting: Tertiary care teaching hospital

Participants: Dengue patient with confirm dengue diagnosis were stratified into two categories on the basis of outcomes i.e. prolonged hospitalization (>3 days) and death. Groups were compared by using appropriate statistical analysis.

Results: Of 667 patients enrolled, 328 (49.2%) had prolonged hospitalization. The mean hospital stay was 4.88±2.74 days. Multivariate analysis showed that DHF (OR 2.3), elevated ALP (OR 2.3), prolonged PT (OR 1.7), aPTT (OR 1.9) and multiple organ dysfunctions (OR 2.1) were independently associated with prolonged hospitalization. Overall case fatality rate was 1.1%. Factors associated with dengue mortality were age >40 years (p=0.004), secondary infection (p=0.040), comorbidities (p<0.05), AKI (p<0.001), prolonged PT (p=0.022), MODs (p<0.001), hematocrit >20% (p=0.001), rhabdomyolosis (p<0.001) and respiratory failure (p=0.007). Approximately, half of the fatal cases in our study also had prolonged hospital stay (>3 days).

Conclusions: The results underscore the high proportion of dengue patients with prolonged hospital stay. Early identification of factors relating to prolonged hospitalization and death will

have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units.

Keywords: Dengue; Hospital stay; Mortality; Risk Factors; Dengue Viral Infection

Strengths and Limitations of this study

- To the best of the authors' knowledge, this is the first study to evaluate predisposing factors of prolonged hospitalization among dengue patients in Malaysia.
- This study involved heterogeneous group of patients from tertiary level teaching hospital of Malaysia, a tropical country with hyperendemic nature of dengue, and hence its findings can be generalized to other tropical regions.
- We analyzed commonly available clinico-laboratory features of dengue patients as predisposing factors that enhances the clinical applicability of current study in medical practice.
- Early recognition of factors identified in current study can potentially improve patient`s outcomes, which in turn can translate to reduced dengue related hospital stay and mortality.
- The admission and discharge criteria may vary among various clinicians attending patients and may interfere with the results of our findings. However, in our institution patients are routinely discharged on the basis of laboratory findings and clinical conditions.

Introduction

Dengue is among the most important arthropod-borne disease that has rapidly been spread in several regions of the world in recent years. The disease is widespread throughout the tropics, with local variations in risk, influence by rainfall, temperature and unplanned rapid urbanization (1). The spectrum of disease varies from mild self-limiting illness, dengue fever (DF) to more severe and fulminating forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (2). The World Health Organization (WHO) estimates that about 40% of world`s population live in areas having risk of dengue transmission. The surge in dengue has been most marked in Asia, with an estimated 1.8 billion people at risk of dengue (3). Southeast Asia in particular has been large epidemics of the disease in recent years with attendant mortality from dengue viral infection (DVI) (4).

Currently, Malaysia is the leading country in terms of the number of dengue cases reported worldwide (5). Dengue is among top five notifiable diseases in the country (6) and continues to be a formidable public health concern. Malaysia is hyperendemic for dengue and experiencing worst dengue crisis nowadays, while some countries in Southeast Asia including Philippines and Thailand have seen decreases in DVI activity in 2014. Reported dengue cases in Malay Archipelago in 2014 increased dramatically (160%) to 98,128 with 189 deaths, compared to 37,698 with 79 deaths in the previous year (7-9). It is worthwhile to mention that year 2015 painted a scary picture, as highest number of dengue cases (N=111,285) along with 301 deaths were ever recorded in the history of the country (Figure 1) (10). More recently, 94 dengue-related deaths have been reported to WHO until April 2016, compared to a total of 120 deaths during the same reporting period in 2015 (3).

Figure 1

The Case Fatality Rate (CFR) due to dengue varies among countries, but can be as high as 10 – 15% in some and <1% in others (11, 12). The recent epidemiology of dengue in Malaysia has imposed substantial economic and disease burden to both patients and health care system in terms of hospital stay, morbidity and mortality. Despite exponential increase in dengue-related deaths in Malaysia, data describing clinico-laboratory characteristics and factors associated with fatal cases are scarce. Efforts made by Sam et al lack statistical comparison between fatal and non-fatal cases and included DHF patients only (13). These findings necessitate the urgent need of studies to differentiate clinico-laboratory characteristics among fatal and non-fatal dengue cases. On the other hand, we previously reported that DVI requires longer hospital stay irrespective of the disease severity resulting in significant burden in terms of health service costs. This is of particular importance in resource limited setting, especially in dengue endemic regions (14). Early identification of risk factors associated with prolonged hospital stay and mortality can help physicians to primacies the management of high-risk dengue patients. These factors could also be utilized to formulate a predicting score to identify severely ill patients in future outbreaks in order to prioritize care and reduce mortality. In this context, a retrospective case series was intended to: (1) compare clinico-laboratory characteristics of fatal and non-fatal dengue patients, (2) compare clinico-laboratory characteristic of patients with and without prolonged hospital stay (>3 days), and (3) identify factors associated with mortality and prolonged hospital stay.

Materials and Methods

Ethical Approval

The study was approved by Human Resource Ethics Committee (JEPeM) of HUSM (USM/JEPeM/14080278). All data was analyzed anonymously and hence, informed consent was not required. The patients were identified from a central computerized record with their registration number (RN). Data of the cases were retrieved and specific numeral codes were given to each case before data analysis. Identity of all patients was not disclosed in current study.

Study location and Population

Current study was conducted in Hospital University Sains Malaysia (HUSM), tertiary level teaching hospital with 950 beds that serves an estimated 1.4 to 1.8 million inhabitants of Kelantan. Kelantan is an agrarian state located in the north-east of Peninsular Malaysia and among top five dengue hotspots in the country where the dengue cases are substantially rising every year. Malays are major (95%) ethnic group in Kelantan while Chinese constitutes merely 4% of state population. The hospital also serves as referral centers for nearby states to treat severe and complicated dengue cases and has reliable medical records (14, 15).

All the dengue patients admitted to the hospital during the period of six years (January 2008 to December 2013) were included into the study. Patients having age ≥ 12 years admitted with primary and confirmed diagnosis of DVI, irrespective of severity, were identified by registration number (RN) using hospital record management system. The process of patient's selection and identification along with inclusion and exclusion criteria are described in Figure 2.

Figure 2

Diagnosis of Dengue

Suspected dengue cases were confirmed by laboratory criteria that were further subjected to clinical case definition of DVI. Suspected dengue infection was defined as the presence of fever and any two of the following symptoms: myalgia, headache, arthralgia, skin rash, retro-orbital pain, hemorrhagic manifestation (s), or leucopenia (white blood cell [WBC] count of $<4\times10^{9}$ L−1). Suspected cases were confirmed by using at least one of the following criteria: (1) positive reverse transcriptase polymerase chain reaction (RT-PCR) result, (2) presence of dengue immunoglobulin M and G antibodies in acute phase serum by enzyme linked immunosorbent assay [Pan Bio Dengue IgM ELISA, Dengue IgM Dot Enzyme Immunoassay, SD Dengue IgM and IgG capture ELISA Kits; Standard Diagnostics, Korea], and (3) at least 4-fold increase of dengue-specific hemagglutination inhibition titers in convalescent serum when compared with acute phase serum. The serum samples were also tested for dengue-specific NS1 [pan-E Early dengue ELISA kit by Panbio, Australia and Platelia dengue NS1Ag assay by Bio-Rad Laboratories, USA). Only confirmed dengue cases were included in analysis. Primary dengue infection was distinguished from secondary infection by using IgM-IgG ratio where dengue infection was defined as primary if ratio ≥ 1.8 and as secondary if < 1.8 or if there was a 4-fold increase of HAI and the titers were ≤1:1280 and ≥1:2560, respectively (14). Serologically confirmed dengue patients were subjected to clinical case definition and disease severity was classified into DF, DHF and DSS, according to the WHO criteria (16). Patient`s demographics and clinical presentations were recorded on day of admission while laboratory findings were recorded for each day of hospitalization until discharge or death, whichever occurred first.

Data Collection and Management

All the required data were collected on structured data collection form, approved by hospital ethical committee. After identification of patients with confirmed dengue, numeral codes were given to patients and these codes were used as identifier during data analysis. Usually patients with dengue infection have hospital stay between 3 or 4 days (14, 17), therefore we used greater than 3 days as cutoff point for prolonged hospitalization (Median hospital stay in present study was 3 days). Patients having hospital stay ≤3 days were compared with those staying >3 days in order to identify possible predictors of increased hospitalization. Similarly, we stratified all patients into fatal and non-fatal cases and their clinical and laboratory characteristics were compared.

Definitions

For the purpose of current study, terms used were defined as follow:

Hospital stay is defined by ≥1 day bed occupancy in hospital; mortality means death within 14 days after admission; hypokalemia (K < 3.5 mmol/L); hyponatremia (Na < 135 mmol/L); oliguria (UO < 400 ml/day after 24 hours of appropriate hydration); hypotension (blood pressure < 110/70 mmHg); elevated transaminases (elevation of liver enzymes such as aspartate aminotransferase [AST] and/or alanine aminotransferase [ALT] >2 times the normal value); transaminitis (elevation of both ALT and AST), prolonged prothrombin time (PT > 15 seconds); prolonged activated partial thromboplastin time (aPTT > 35 seconds); urinary sedimentations (presence of glycosuria, hematuria, proteinuria, leucocytouria, urine pus, urine epithelial cells); anemia (Hb < 12 g/dL); dengue viral infection (dengue fever, dengue hemorrhagic fever, dengue shock syndrome); Acute kidney injury (AKI) (Acute Kidney Injury Network (AKIN) of classification); stages of AKI based upon serum creatinine values (AKIN-I, AKIN-II, AKIN-III); severe dengue (DHF, DSS); multiple organ dysfunction (dysfunction of ≥2 organs); hepatic

dysfunction (elevation of liver enzymes); and thrombocytopenia (platelets count < 100×10^9 cells). Reference values of laboratory parameters in current study are according to the hospital pathology lab included AST (5-34 IU/L); ALT (10-35 IU/L); ALP (♂: 53-168 ♀: 42-98 IU/L); Hematocrit (♂:37.5-49.8 ♀: 31.8-42.4); Platelets (158-410×10^9/L); WBCs (♂:3.8-9.7 ♀: 3.4-10.1); PT (12-13 seconds); aPTT (30-50 seconds).

Statistical analysis

All the patients were divided into two groups based upon presence or absence of outcomes (mortality, prolonged hospital stay). For quantitative variables, measures of central tendency and dispersion were calculated. Qualitative variables are presented as frequencies and proportions for which frequency was served as numerator and total number of patients (n=667) was served as denominator. Relevant denominator was stated before proportion, where it varied. Comparison of categorical variables between two groups was done by using Chi-Square test (if at least 80 percent of cells have expected frequencies of 5 or more) or Fisher`s Exact test (if less than 80 percent of cells have expected frequencies of 5 or more). Comparison of continuous variables was done by an independent Student's t-test. Logistic regression was used to estimate the associations between prolonged hospital stay, as the response variables, and potential predictors. These potential predictor variables were chosen on the basis of statistical significance and their biological plausibility with the outcomes. Co-linearity diagnostics was performed on variables selected for regression analysis. The strength of association was evaluated using an odds ratio (OR) and a 95% confidence interval (CI). The variables in univariate analysis with p value less than 0.25 were subjected to multivariate analysis (18). The use of univariate *P* values <0.25 has advantage of tending to include more variables in multivariate analysis while traditional levels of *P* value such as 0.05 can fail in identifying variables known to be important

(19). Calibration of final multivariate logistic model (model fit) was assessed by Hosmer-Lameshow test. The two-sided statistical significance level, p-value, was set at 0.05 for all inferential analyses in this study. Data were compiled and analyzed using Statistical Package for Social Sciences program version 20 (SPSS: Inc. Chicago. Il. USA).

Results

Out of the total dengue cases admitted to the hospital, 667 patients with mean age 30.8 ± 16.1 years were included in analysis (Figure 2). According to WHO criteria, DF was diagnosed in 627 (88.1%) patients while DHF (DHF grade I & II) and DSS (DHF grade III & IV) were observed in 74 (11.1%) and 5 (0.8%) cases, respectively. Most of the studied participants were ethnic Malay (90.4%) followed by Chinese (7.9%) and Indians (1.4). The most common non-hemorrhagic manifestations at hospital admission were fever (97%), headache (58.9%), retro-orbital pain (25%), Myalgia (70.4%), arthralgia (56.5%), nausea (31.2%), vomiting (54.2%), abdominal pain (44.7%), cough (18.5%), chills (31.5%), anorexia (27%), malaise (7.3%), lethargy (27.4%), palpitation (2%), flushing (19%), dehydration (11%), ascites (2.7%) and pleural effusion (3.2%). On the other hand, petechia (11.2%), hematemesis (1.9%), purpura ecchymosis (7.4%), epistaxis (4.9%), hematuria (2.5%) and gum bleeding (9.4%) were common hemorrhagic signs and symptoms among dengue patients.

The mean length of hospital stay (LOS) was 4.88 ± 2.74 days (median 3, IQR 3, range: 1-35 days). Prolonged hospitalization (>3 days) were seen in 49.2% (328/7667) patients while length of stay (LOS) was ≤3 days among 50.8% (339/667) studied participants. Clinical characteristics among patients with and without prolonged LOS were compared (Table 1). Patients having DHF, hypertension (HTN), elevated ALP, multiple organ dysfunctions (MODs), prolonged PT and aPTT were signicantly associated with prolonged hospitalization. Although, other factors such as secondary infection, comorbidities, acute kidney injury (AKI), elevated AST and hematocrit > 20% were more profound among patients with prolonged hospitalization

but their statistical association was insignificant (P>0.05). It was interesting to note that male gender and patients with DF were more likely to stay ≤ 3 days in current study (Table 1).

Table 1

To determine the factors independently associated with prolonged hospitalization, we developed a series of logistic regression analyses, which are shown in Table 2. Out of eight readily available clinical parameters that had p-values <0.25 in the univariate analysis, four factors (DHF, elevated ALP, increased PT/aPTT, MODs) were found to be independently associated with prolonged length of stay (LOS). Although, presence of HTN and thrombocytopenia were found as risk factors of prolonged LOS in unadjusted analysis but neither of these showed statistically significant results in multivariate model. Though, thrombocytopenia and AKI were insignificant variables in Table 1 but included in logistic regression due to their hypothetical and clinical association with prolonged hospitalization. ROC curve analysis with AUC as 0.812 demonstrated that logistic model has good predictive ability for prolonged hospitalization (Figure 3).

Table 2

Figure 3

Evaluation of Dengue related Fatal cases

The overall dengue case fatality rate (CFR) was 1.1% and all eight fatal cases were attributed to the dengue infection. Of the dengue deaths, 4 (50%) were male, 6 (75%) were Malay and two (25%) were Chinese. The mean age was 48.8 ± 25.6 years (range: 13-78) and most of the fatal cases (n=6/8, 75%) were rural residents. DF was present in 6/8 (75%) patients while DHF was

recorded in 2/8 (25%) cases. Four patients (50%) had no pre-existing comorbidities and remaining four patients (50%) were having at least two comorbid conditions (Table 3).

The mean duration of hospital stay among death cases was 5.6 ± 3.2 days (median: 5, IQR: 9, range 3-12 days) and all of them were admitted ≥5th day of onset of illness. The number of fatal cases in present study was very small that precluded us to perform logistic regression analysis. However, results of Chi-square test demonstrated significantly higher proportion of age >40 years, secondary infection, comorbidities (DM, IHD), AKI, prolonged PT, MODs, hemoconcentration, rhabdomyolosis and respiratory failure among death cases as compared to patients who survived (Table 3).

Table 3

The main presenting complaints in all fatal cases are shown in Table 4. Fever, nausea, vomiting and abdominal pain were presented in all death cases while retro-orbital pain, dysuria and shortness of breath (SOB) was present in 6/8 (75%) patients.

Table 4

Table 5 showed individual data points of all eight patients who died. All of them were brought alive to the hospital and succumbed to the infection within 3-12 days of admission. These patients were admitted on or after day 5 of illness and had rapid deterioration of their clinical features resulting admission to the intensive care unit (ICU). Dengue infection was confirmed in all patients by dengue serological tests. Three patients (P1, P2 & P3) had clinical history relating to dengue fever. About one third patients died within 72 hours of hospital admission. On admission, all the cases were febrile (mean 38.9 ^{o}C) and about 50% patients had

pulse rate >100 beats per minute (BPM). We observed multifactorial causes of death in current study including dengue infection complicated with multiple organ dysfunctions (100%), acute kidney injury (100%), shock (25%), acute respiratory distress syndrome (25%), disseminated intravascular coagulation (12.5%), gastric bleeds (25%) and underlined comorbid conditions (50%). Five died patients (62.5%) had prolonged hospital stay in current study. Unfortunately, we had no access to postmortem and autopsy data of fatal cases.

Table 5

Discussion

The alarming rise of dengue epidemiology has been highlighted to haunt 40% of world population. In Malaysia, dengue is perceived as a highly contagious health threat with escalating trend of infection. The average number of dengue cases and death tolls had recorded a high surge over the past few years leading to substantial disease burden in terms of cost. Of the estimated, the annual cost for dengue illness (standard errors in parenthesis) in Malaysia is US$42.4 (±4.3) including per capita cost US$4.73 for one thousand population size (n=100) with disability adjusted life years (DALYs) equivalent to 8,324 (20). As stated earlier, dengue induced disease burden related to cost of care and mortality is of particular importance, especially in developing countries where the dengue is endemic (15). Early identification of dengue patients having high risk of prolonged hospital stay and death may serve as an effective tool to combat the upsurge of disease burden. In this attempt, we evaluated several factors associated with prolonged hospitalization (≥3 days) and mortality among dengue patients attending tertiary care hospital.

Approximately half of the studied participants had prolonged hospitalization, indicating the substantial burden in terms of cost of care to both patients and health care system. In our recent series, we reported that prolonged hospital stay is not only associated with DHF but also with classical DF (14). The proportion of prolonged hospitalization in DF and DHF was 46.7% (n=275/588) and 67.1% (n=53/79), respectively. These findings are in agreement with previous studies describing association of dengue infection with prolonged hospitalization, irrespective of severity (14, 15, 21). We found statistical association of DHF and prolonged hospital stay in present study, where individuals suffering from DHF had twice higher odds of longer hospital stay than patients without DHF. The mean hospital stay in present series (4.47

days) is consistent with previous investigations reporting mean hospitalization between 3.4 to 6.2 days (14, 21-24). We found that dengue patients with hypertension, elevated ALP, prolonged PT/aPTT and multiple organ dysfunctions (MODs) were strongly associated (P<0.005) with hospital stay >3 days (Table 1). These findings are in concordance with the results of one and only study evaluating predictors of longer hospitalization among dengue patients (21).

Many risk factors were identified and evaluated independently in their contribution to prolonged hospitalization (Table 2). Of these, presence of DHF, elevated ALP, prolonged PT/aPTT and MODs were found to be predisposing factors of longer hospital stay. Though, Khalil *et al.* reported old age and AKI as predictors of increased hospital stay but present study did not show any statistical association of these variables with length of stay (21). Age was equally distributed among patients with and without longer hospital stay in our study (Table 1). It might be due to population differences, as Khalil and colleagues included patients aged >14 years while we included patients with age ≥12 years in our study. Several previous case series have demonstrated the association of elevated SCr or AKI with prolonged hospitalization and death (14, 15, 21, 25, 26). Although, prevalence of AKI in our study was high (14.2%) but was equally distributed (P=0.242) among patients with and without prolonged hospitalization (Table 1). However, subgroup analysis showed that patients with AKI had signicantly (p<0.001) longer duration of hospital stay as compared to patients without AKI. Our findings, in addition to several other studies reporting the impact of elevated Scr on prolonged hospital stay and death, suggest the monitoring of Scr while managing dengue patients.

Significant number of patients in our study had coagulopathy attributed to low platelets, deranged PT, aPTT and elevated liver transaminases (Table 1). More than half of the study participants with prolonged PT and aPTT were associated with longer hospitalization in current

study (Table 1). Involvement of liver has been well documented during the course of dengue infection (27). However, only elevated ALP was observed as an independent predictor of longer hospital stay. Moreover, dysfunction of several other organs included brain, heart, muscles, spleen, and gallbladder has recently been emerged as expanded dengue syndrome (EDS) and might be contributed directly to viral localization in organs or to development of ischemia of various organs as a result of AKI, coagulopathy and old age (16, 28). Dengue patients with multiple organ dysfunctions (MODs) had two times higher risk of longer hospital stay in present study. Notably, DHF in combination with MODs, hypertension, coagulopathy and elevated ALP might denote seriously sick patients who can potentially have more morbidity in the form of increased hospital stay. Identification of these patients at the earliest and their management with special care would be advantageous in reducing morbidity and hence their bed occupancy in the hospital.

Dengue viral infections are rarely fatal, although fatal infections do occur due to plasma leakage, fluid accumulation, respiratory distress, severe bleeding or MODs (14). Overall mortality in current study was 1.1% that is consistent with previous national (13, 14) and global studies (21, 26, 29). Malaysia experienced large dengue epidemics in the past decade affecting predominantly adults (5). This shift from childhood to adult illness is attributed to lower herd immunity and transmission outside home (5, 14). Though, current study included patients with age >12 years but we did not observe any mortality among patients aged <12 years during the study period (data is not shown). Out of eight died cases, two patients (P1 & P4) belonged to adolescent age group (14 and 13 years) while remaining had advanced age above 40 years (Table 3 & 5). Interestingly, dengue patients with age >40 years were significantly associated with mortality in current study. The higher risks of dengue related deaths with increasing age might be

contributed to decline in physiological functions and underline diseases in aging people. In addition to comorbidities, older patients would have rehabilitation issues which may complicate admission and extend hospital stay. Dengue patients with age >50 years were reported to be at high risk of hospitalization and mortality in two separate series (30, 31). Prolonged hospitalization as defined in current study was observed in 50% (n=4/8) of fatal cases. Our study explicitly illustrates that advanced age is also associated with increased length of hospitalization, in addition to high mortality rate. These findings are consistent with Taiwanese study suggesting the impact of increasing age with higher fatality and longer hospitalization (32). On the other hand, it can also be assumed that dengue patients with advanced age staying more than 3 days in the hospital should be considered as high risk patients for mortality. However, we recommend large multicentric controlled studies to evaluate this assumption. On the other hand, we found equal distribution of gender among fatal cases (Table 3) and this is in contrast with the findings of Malaysian study reporting preponderance of dengue deaths amongst females (13).

It is interesting to note that all those patients who died had severe AKI and multiple organ dysfunctions (MODs) (Table 3 & 5). These findings are in agreement with the results of Khalil *et al*. who reported AKI in all fatal cases (21). In another case series, Lee *et al.* reported the presence of AKI in 80% patients who died (32). Moreover, AKI as a cause of death has been documented in two separate studies reporting prevalence of AKI in 30% and 14% of fatal cases (13, 29). We also presented similar findings where severe AKI was among documented causes of death (Table 5). This study also found that secondary infection, diabetes mellitus, IHD, prolonged PT, hematocrit >20%, rhabdomyolosis and respiratory failure was associated with dengue-related mortality in the overall study population (Table 3). Current data also suggest that rhabdomyolosis and coagulation disorders are not uncommon intricacies in dengue infection,

implicating that clinicians should be alert to the possible occurrence of these complications when caring dengue patients.

Secondary infection usually occurs in dengue endemic regions and correlates to disease severity (15). Three of the fatal cases in this series had secondary infection while remaining five cases had evidence of primary infection (Table 5). Although, this observation is in contrast to the other reports illustrating occurrence of mortality predominantly in secondary infection (11, 13, 30) but in agreement with the findings of Ong et al where 3 out of 7 death cases had primary infection (29).

Mortality is usually linked to delayed provision of supportive treatment and/or pre-morbid chronic illness (14, 16). All died cases were admitted on day five (range: 5 – 8 days) of illness and most of them had defervescence, followed by rapid deterioration of clinical condition. In our study, the average duration of illness prior to hospitalization among death cases was significantly differ from those who survived (P=0.025). Our observation is consistent with earlier studies done on dengue deaths where late hospitalization was found to be a possible contributing factor to increased risk of mortality (13, 29, 11). These findings suggest that early care-seeking behavior may determine the possibility of receiving and thereby avoiding fatal outcomes.

Besides care-seeking behavior pattern, the presence of comorbidities is of paramount importance among dengue patients. Several reports suggested that worsening of co-morbid conditions, rather than directly from dengue infection could be the reason for death seen especially in adults (33, 34). Half of the death cases in present study had underlying comorbid illness including CKD, DM, HTN and IHD. Patients with diabetes and IHD had higher risks of death, when compared to their survived counterparts (Table 3). Several earlier reports implicated

DM as a possible contributing factor to death (33, 34). Nonetheless, 50% death cases were healthy individuals without any comorbid condition. This observation highlighted that underlying co-morbidities might not be the main factor contributed to the dengue mortality in our cohort.

Good early predictors of mortality are presently lacking. Several reports emphasized the importance of individual warning signs as tool to recognize patients having high risk of severe illness and death (11, 34, 35). All the fatal cases in our study had at least one warning sign (Table 4) and these findings are consistent with Cuban study reporting presence of warning signs in all twelve fatalities (11). Contrary to these findings, Ong *et al.* reported presence of warning signs in 50% of death cases (29). Hepatomegaly is usually associated with severe dengue (36) and was observed in two patients in our series. Notably, hepatomegaly was not only seen in DHF (Patient 2, Table 4) but also in DF (Patient 5, Table 4). Hepatospleenomegaly, usually associated with macrophage activation syndrome (MAS), was present in only one patient (Patient 2, Table 4) suggesting its association with dengue infection. Such association has also been previously reported in the literature (37). These observations underscore that attention should be paid to uncommon presentations because they may often related to poor prognosis and high mortality. The causes of death in current study are consistent with those reported in other series on dengue fatal cases (11, 13, 38).

This retrospective study identified several factors associated with prolonged hospital stay and mortality among dengue patients attending tertiary care hospital. These factors should be identified at the earliest and treated preferably in a special care setup. Use of advanced therapies (renal replacement, ventilator support) along with inotropes or antibiotics (for

hemodynamic disturbances and sepsis), can potentially improve patient`s outcomes, which in turn can translate to reduced dengue related hospital stay and mortality.

Study Limitations

However several shortcomings of the present study should be considered. This study is a single-center study with retrospective analysis and the results may therefore not necessarily be generalized to other populations. Additionally, study depends on thoroughness of clinician`s documentation so disease severity and clinical outcomes of included patients may be biased due to lack of standardized management protocol for dengue. The retrospective nature of data collection may have biased the findings towards patients who are more unwell and hence more likely to be recorded and captured by the system. The admission and discharge criteria may vary among various clinicians attending patients and may interfere with the results of our findings. The small number of fatal cases in our study may make statistical power quite small for identification of factors associated with dengue-related mortality. However, four case series (32, 29, 38, 39) with small number of the fatal cases (n=7, n=5, n=19, n=18) evaluating factors of mortality in dengue infection are in support of current study. A case control study with much larger sample size is needed to determine if those common clinical and laboratory findings seen in our series are exclusive to fatal cases of dengue or seen equally in non-fatal cases. Moreover, study population included adults and hence results cannot be generalized to pediatric patients. Unfortunately, autopsy and postmortem data of these patients were not available. Last but not least, WHO 1997 criteria of dengue classification were used in current study because similar criteria are being used in Malaysia. A recent investigation has also verified diagnosis of dengue using WHO 1997 classification in Malaysia. However, health authorities included WHO 2009 criteria in new guidelines on dengue infection that issued in November, 2016.

Nevertheless, the strength in this study lies in our attempt to include large heterogeneous dengue population. Present study improves awareness of factors associated with prolonged hospital stay and death among dengue patients. It also highlights the need for more studies and for strategized management protocol in order to reduce disease burden in the form of cost or death. Moreover, this is only largest study conducted in Malaysia describing factors relating to prolonged hospitalization. These observations along with our previous case series (14, 15, 18, 25, 28) prove to be a valuable addition in dengue literature and warrants further investigations.

Conclusion

In conclusion, our case series demonstrates high proportion of dengue patients with prolonged hospital stay. Patients with severe disease (DHF) along with elevated ALP levels and deranged PT/aPTT had higher likelihood to stay more than 3 days in the hospital. Our observations exemplified that fatal dengue infection does occur in adults and in primary infection, irrespective to gender. Factors including advance age, secondary infection, comorbidities, prolonged PT, hemoconcentration and rhabdomyolosis were found to be statistically associated with mortality. Early identification of dengue patients with these factors will have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units. Attention to these high risk patients may have a positive impact on both patients and health care system by reducing dengue related bed occupancy and mortality.

Recommendation

For clinical practice, doctors treating dengue especially those working in the front-line should be made aware of the factors significantly associated with longer hospital stay and

dengue mortality. With proper identification of these factors, time and resources can be focused on those at highest risk.

Conflict of interest: Authors declare no conflict of interest.

Funding: There is no funding for this project.

Author`s contribution: THM, YHK and ASA provided substantial contributions to the conception or design of the work. THM and AS were involved in the acquisition analysis and interpretation of data. THM and AHK were responsible for drafting the work or revising it critically for important intellectual content. AHK and ASA gave final approval of the version to be published. All the authors substantially contributed to the manuscript and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Sharing: All the relevant data are within manuscript.

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Figure Legends

Figure 1: Incidence of dengue cases and deaths in Malaysia (Ministary of Health Malaysia, epidemiological data of WHO and MOH may slightly vary due to the differences in reporting criteria or lack of reporting)

Figure 2: Methodological Flow of Study

Figure 3: ROC curve analysis of multivariate logistic model predicting prolonged hospitalization among dengue patients (AUC: area under the curve, CI: confidence interval)

Table 1: Comparison of clinical characteristics (on-admission) of dengue patients according to the presence or absence of prolonged hospital stay (>3 days)

Variables	Total cohort N=667	LOS in hospital		P values
		≤ 3 days (N=339)	>3 days (N=328)	
Age (years), mean±SD	30.8 ± 16.1	30.7 ± 15.8	30.7 ± 16.5	0.305
Age >40 yrs, n (%)	167 (25%)	84 (24.5%)	83 (25.3%)	0.875
Age >60 yrs, n (%)	35 (5.2%)	17 (5.1%)	18 (5.5%)	0.784
Male gender, n (%)	378 (56.7%)	189 (55.8%)	139 (42.4%)	0.626
Secondary infection, n (%)	73 (10.9%)	33 (9.7%)	40 (12.2%)	0.309
Dengue severity, n (%)				
DF, n (%)	588 (88%)	313 (92.3%)	275 (83.8%)	0.001
DHF[§], n (%)	79 (10.5%)	26 (7.7%)	53 (16.2%)	**0.001**
Warning signs, n (%)	271 (40.6%)	141 (41.6%)	130 (39.6%)	0.607
Comorbidities, n (%)				
DM, n (%)	36 (5.4%)	14 (4.1%)	22 (6.7%)	0.141
HTN, n (%)	35 (5.2%)	12 (3.5%)	23 (7%)	**0.044**
CKD, n (%)	33 (4.9%)	15 (4.4%)	18 (5.5%)	0.522
IHD, n (%)	25 (3.7%)	9 (2.7%)	16 (4.9%)	0.131
Comorbidities ≥2, n (%)	36 (5.4%)	13 (3.8%)	23 (7%)	0.069
AKI, n (%)	95 (14.2%)	43 (12.7%)	52 (15.9%)	0.242
Scr ≥ 2mg/dL, n (%)	29 (4.1%)	10 (2.9%)	19 (5.8%)	0.048
Elevated ALT, n (%)	362 (54.3%)	192 (56.6%)	170 (51.8%)	0.197
Elevated AST, n (%)	447 (67%)	226 (66.7%)	221 (67.4%)	0.636
Elevated ALP, n (%)	133 (19.9%)	44 (13%)	89 (27.1%)	**<0.001**
Prolonged PT, n (%)	224 (33.3%)	80 (23.6%)	144 (43.9%)	**<0.001**
Prolonged aPTT, n (%)	159 (23.8%)	52 (15.3%)	107 (32.6%)	**<0.001**
Thrombocytopenia, n (%)	395 (59.2%)	191 (56.3%)	204 (62.2%)	0.134
MODs, n (%)	117 (17.5%)	44 (13%)	72 (22.3%)	**0.002**
Hematocrit > 20 %, n (%)	73 (10.9%)	38 (11.2%)	35 (10.7%)	0.824

[§]DHF includes all four grades

P values were calculate between patients with and without prolonged hospital stay

Abbreviations: LOS: length of stay, DF: dengue fever, DHF, dengue hemorrhagic fever, DSS: dengue shock syndrome, DM: diabetes mellitus, HTN: hypertension, CKD: chronic kidney disease, IHD: ischemic heart disease, AKI: acute kidney injury, Scr: serum creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, MODs: multiple organ dysfunction

Table 2: Univariate and Multivariate analysis to evaluate determinants (risk factors) of prolonged length of hospital stay (LOS)

Variables*	Univariate analysis			Multivariate analysis		
	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**
DHF	0.001	2.3	1.4 to 3.8	**0.009**	2.3	1.2 to 4.3
HTN	0.048	2.1	1.1 to 4.2	0.688	1.2	0.5 to 2.1
Elevated ALP	<0.001	2.5	1.7 to 3.7	**<0.001**	2.3	1.5 to 3.5
Prolonged PT	<0.001	2.6	1.9 to 3.6	**0.009**	1.7	1.1 to 2.5
Prolonged aPTT	<0.001	2.7	1.8 to 3.9	**0.005**	1.9	1.2 to 2.9
Thrombocytopenia	0.135	1.3	0.9 to 1.7	0.197	1.3	0.9 to 1.8
AKI	0.242	1.3	0.8 to 2.0	0.174	0.7	0.3 to 1.2
MODs	0.002	1.9	1.3 to 2.9	**0.013**	2.1	1.2 to 3.7

*Variables lack multi-collinearity and had variance inflation value (VIF) less than 2
Hosmer-Lameshow Test Statistics: Chi-square: 7.474, degree of freedom: 6, p value = 0.279
Abbreviations: DHF: dengue hemorrhagic fever, HTN: hypertension, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, AKI: acute kidney injury, MODs: multiple organ dysfunction

Table 3: Comparison of clinical characteristics (on-admission) of fatal and non-fatal dengue cases

Variables	Total cohort N=667	Non-Fatal Cases N = 659	Fatal cases N = 8	P* values
Age (years), mean±SD	30.7 ± 16.1	30.5 ± 1597	48.8 ± 25.6	0.083
Age >40 yrs, n (%)	167 (25%)	161 (24.4%)	6 (75%)	**0.004**
Age>60 yrs, n (%)	35 (5.2%)	33 (5.0%)	2 (25%)	0.061
Male gender, n (%)	378 (56.7%)	374 (56.8%)	4 (50%)	0.732
Secondary infection, n (%)	73 (10.9%)	70 (10.6%)	3 (37.5%)	**0.047**
Dengue severity, n (%)				
DF, n (%)	588 (88%)	582 (88.3%)	6 (75%)	0.243
DHF[§], n (%)	79 (10.5%)	77 (11.7%)	2 (25%)	0.243
Comorbidities, n (%)				
DM, n (%)	36 (5.4%)	33 (5.0%)	3 (37.5%)	**0.007**
HTN, n (%)	35 (5.2%)	33 (5.0%)	2 (25%)	0.061
CKD, n (%)	33 (4.9%)	31 (4.7%)	2 (25%)	0.055
IHD, n (%)	25 (3.7%)	23 (3.5%)	2 (25%)	**0.033**
Comorbidities ≥2, n (%)	36 (5.4%)	33 (5.0%)	4 (50%)	**0.007**
AKI, n (%)	95 (14.2%)	87 (13.2%)	8 (100%)	**<0.001**
Scr ≥ 2mg/dL, n (%)	29 (4.1%)	21 (3.2%)	8 (100%)	**<0.001**
Elevated ALT, n (%)	362 (54.3%)	360 (54.6%)	2 (25%)	0.151
Elevated AST, n (%)	447 (67%)	443 (67.2%)	4 (50%)	0.112
Elevated ALP, n (%)	133 (19.9%)	133 (20.2%)	0	-
Prolonged PT, n (%)	224 (33.3%)	218 (33.1%)	6 (75%)	**0.022**
Prolonged aPTT, n (%)	159 (23.8%)	157 (23.8%)	2 (25%)	1.000
Thrombocytopenia, n (%)	395 (59.2%)	393 (59.6%)	2 (50%)	0.072
MODs, n (%)	117 (17.5%)	109 (16.5%)	8 (100%)	**<0.001**
Hematocrit > 20 %, n (%)	73 (10.9%)	68 (10.3%)	5 (62.5%)	**0.001**
Rhabdomyolosis, n (%)	49 (6.9%)	42 (6.4%)	7 (87.5%)	**<0.001**
Respiratory Failure, n (%)	11 (1.5%)	9 (1.4%)	2 (25%)	**0.007**

[§]DHF refers to all grades including 5 cases of DSS
*P values were calculate between patients with and without prolonged hospital stay
LOS: length of stay, DF: dengue fever, DHF, dengue hemorrhagic fever, DSS: dengue shock syndrome, DM: diabetes mellitus, HTN: hypertension, CKD: chronic kidney disease, IHD: ischemic heart disease, AKI: acute kidney injury, Scr: serum creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, MODs: multiple organ dysfunction

Table 4: Clinical Features (presenting complaints) of Dengue Fatal Cases (n=8)

Sign and Symptoms	P1	P2	P3	P4	P5	P6	P7	P8	n (%)
Fever	×	×	×	×	×	×	×	×	8 (100%)
Nausea	×	×	×	×	×	×	×	×	8 (100%)
Vomiting	×	×	×	×	×	×	×	×	8 (100%)
Abdominal pain	×	×	×	×	×	×	×	×	8 (100%)
Retro-orbital pain	×	×	×	×		×	×		6 (75%)
Dysuria	×	×		×	×		×	×	6 (75%)
Chills	×		×	×		×			4 (50%)
Shortness of breath	×	×		×	×		×	×	6 (75%)
Headache		×	×			×	×		4 (50%)
Myalgia		×				×	×	×	4 (50%)
Diarrhea	×	×		×			×		4 (50%)
Malaise		×	×			×	×		4 (50%)
Lethargy		×			×		×	×	4 (50%)
Restlessness					×			×	2 (25%)
Jaundice			×		×	×		×	4 (50%)
Rigors		×	×			×	×		4 (50%)
Pleural effusion	×		×	×		×			4 (50%)
Skin rash	×	×		×			×		4 (50%)
Dizziness				×				×	2 (25%)
Anorexia		×					×		2 (25%)
Confusion		×					×		2 (25%)
Asthenia					×			×	2 (25%)
Petechia/ Purpura	×	×							2 (25%)
Gum bleeding	×	×							2 (25%)
Spleenomegaly		×		×					2 (25%)
Hepatomegaly		×			×				2 (25%)
Conjunctivitis			×				×		2 (25%)
Edema	×			×					2 (25%)
Ascites	×		×						2 (25%)
Anascara	×			×					2 (25%)
All the signs and symptoms listed were presented at hospital admission P1 to P8 demonstrated patients numbers from 1 to 8									

Table 5: Demographics, co-morbidities, clinical features and causes of death of fatal dengue patients seen at HUSM between 2008 to 2013

Patients	Age (years) /gender	Duration of illness prior to admission (day)	Secondary infection	Comorbidities	Dengue diagnosis	Duration of hospital stay/duration of fever	Complications/ causes of death*
P1	14 / M	6	Yes	Nil	DHF	4 / 2	DHF complicated with MODs, severe AKI, rhabdomyolosis, respiratory failure, bleeding tendencies, ARDS
P2	78 / F	7	No	IHD, HTN, newly diagnosed OP	DF	6 / 4	Septic shock, probable leptospirosis, DF complicated with MODs, severe AKI, septicemia, rhabdomyolosis, bleeding tendencies, IHD, HTN
P3	43 / F	8	No	Nil	DF	8 / 2	Severe AKI, DF complicated with MODs, rhabdomyolosis, DIC, gastric disturbances, hypotension
P4	13 / M	5	No	Nil	DF	3 / 2	DF complicated with MODs, severe AKI, dehydration secondary to dengue fever, rhabdomyolosis, respiratory failure, ARDS
P5	59 / M	6	No	CKD, DM	DF	3 / 2	Acute on chronic renal failure, severe AKI, DF complicated with MODs, rhabdomyolosis, DM, CKD
P6	44 / F	5	No	Nil	DF	12 /2	Severe AKI , DF complicated with MODs, DIC, dehydration secondary to dengue fever, gastric disturbances, hypotension
P7	77 / F	6	Yes	IHD, HTN, DM	DHF	7 / 4	Septic shock, DHF complicated with MODs, severe AKI, septicemia, rhabdomyolosis, acute myocardial infraction, IHD, HTN
P8	60 / M	5	Yes	DM, CKD	DF	3 / 2	Renal complications, acute on chronic renal failure, DF complicated with MODs, severe AKI, ketoacidosis, rhabdomyolosis, DM, CKD
P1 to P8: number of patients from 1 to 8, F: female, M: male							

Figure 1

	Cases	Deaths
1995	6534	28
1996	14255	32
1997	19429	52
1998	27381	82
1999	101146	37
2000	7103	45
2001	16368	50
2002	32767	99
2003	31545	72
2004	33895	102
2005	39654	107
2006	38556	92
2007	48846	98
2008	49335	112
2009	41486	88
2010	46171	134
2011	19884	36
2012	21900	35
2013	43346	92
2014	108698	215
2015	120836	336

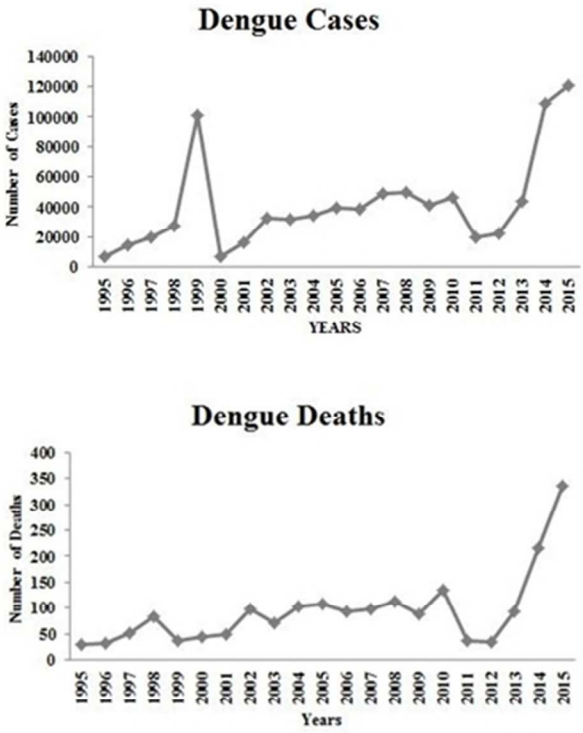


Figure 2

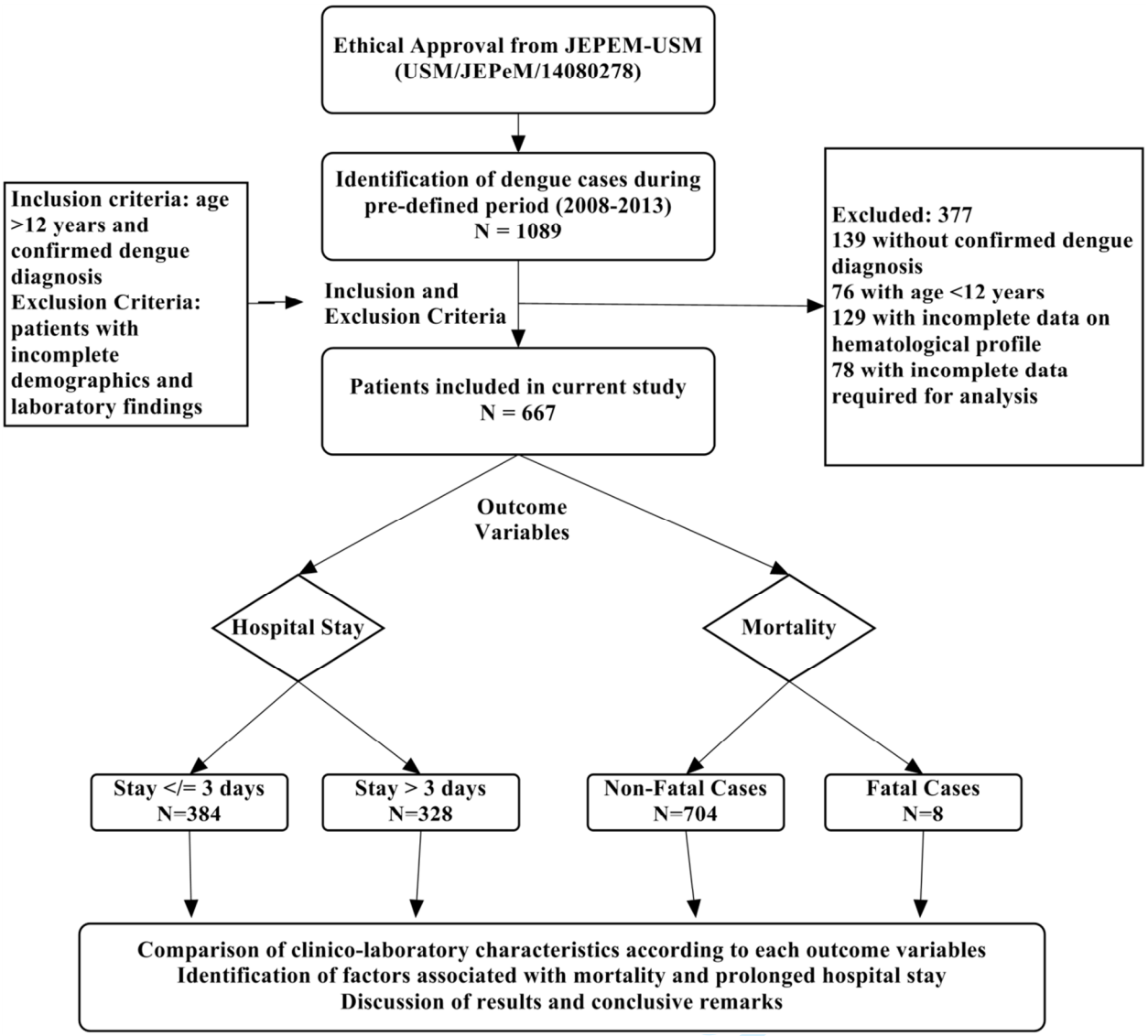
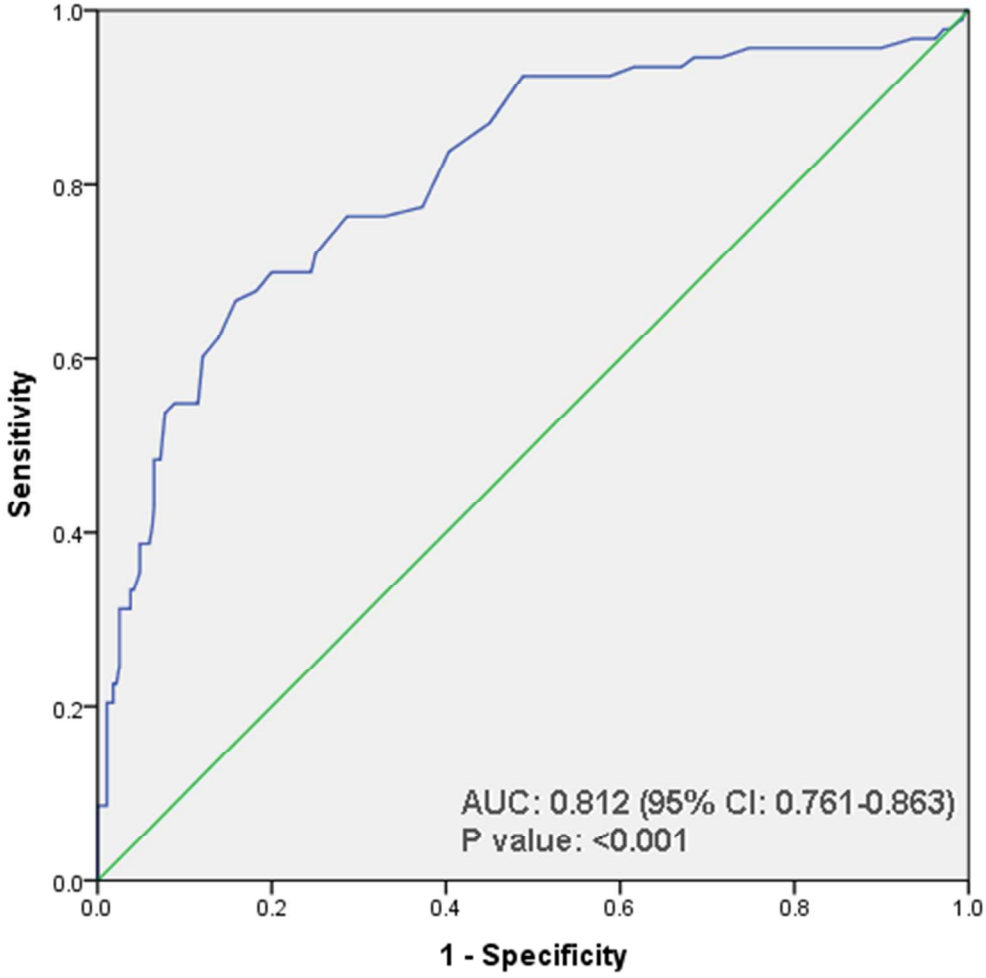


Figure 3



STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	9, 10
		(*c*) Explain how missing data were addressed	9, 10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	9, 10

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9, 10
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	13
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14
		(*b*) Report category boundaries when continuous variables were categorized	13, 14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13, 14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13, 14
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-23
Generalisability	21	Discuss the generalisability (external validity) of the study results	22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	n/a

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
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BMJ Open

Determinants of Mortality and Prolonged Hospital stay among Dengue patients attending Tertiary Care Hospital: A Cross-Sectional Retrospective Analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-016805.R1
Article Type:	Research
Date Submitted by the Author:	09-May-2017
Complete List of Authors:	Mallhi, Tauqeer; University Sains Malaysia, Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences; khan, Amer hayat Sarriff, Azmi; University Sains Malaysia, Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences Adnan, Azreen; University Sains Malaysia, Chronic Kidney Disease Resource Centre, School of Medical Sciences, Health Campus Khan, Yusra; University Sains Malaysia, Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	Dengue, Hospital stay, Mortality, Risk Factors, Dengue Viral Infection, Dengue Hemorrhagic Fever

Determinants of Mortality and Prolonged Hospital stay among Dengue patients attending Tertiary Care Hospital: A Cross-Sectional Retrospective Analysis

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Running title: Dengue related prolonged hospital stay and mortality

Keywords: Dengue; Hospital stay; Mortality; Risk Factors; Dengue Viral Infection; Dengue Hemorrhagic fever

Word count: 4238 words

Abstract

Objectives: Dengue imposes substantial economic, societal and personal burden in terms of hospital stay, morbidity and mortality. Early identification of dengue cases with high propensity of increased hospital stay and death could be of value in isolating patients in need of early interventions. Current study was aimed to determine the significant factors associated with dengue-related prolonged hospitalization and death.

Design: Cross-sectional retrospective study

Setting: Tertiary care teaching hospital

Participants: Dengue patients with confirm diagnosis were stratified into two categories on the basis of prolonged hospitalization (≤ 3 days and >3 days) and mortality (fatal cases and non-fatal cases). Clinico-laboratory characteristics between these categories were compared by using appropriate statistical methods.

Results: Of 667 patients enrolled, 328 (49.2%) had prolonged hospitalization. The mean hospital stay was 4.88±2.74 days. Multivariate analysis showed that DHF (OR 2.3), elevated ALP (OR 2.3), prolonged PT (OR 1.7), aPTT (OR 1.9) and multiple organ dysfunctions (OR 2.1) were independently associated with prolonged hospitalization. Overall case fatality rate was 1.1%. Factors associated with dengue mortality were age >40 years (p=0.004), secondary infection (p=0.040), comorbidities (p<0.05), AKI (p<0.001), prolonged PT (p=0.022), MODs (p<0.001), hematocrit >20% (p=0.001), rhabdomyolosis (p<0.001) and respiratory failure (p=0.007). Approximately, half of the fatal cases in our study had prolonged hospital stay of greater than days.

Conclusions: The results underscore the high proportion of dengue patients with prolonged hospital stay. Early identification of factors relating to prolonged hospitalization and death will have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units.

Keywords: Dengue; Hospital stay; Mortality; Risk Factors; Dengue Viral Infection; Dengue Hemorrhagic fever

Strengths and Limitations of this study

- To the best of the authors' knowledge, this is the first study to evaluate predisposing factors of prolonged hospitalization among dengue patients in Malaysia.
- This study involved heterogeneous group of patients from tertiary level teaching hospital of Malaysia, a tropical country with hyperendemic nature of dengue, and hence its findings can be generalized to other tropical regions.
- We analyzed commonly available clinico-laboratory features of dengue patients as predisposing factors that enhances the clinical applicability of current study in medical practice.
- Early recognition of factors identified in current study can potentially improve patient`s outcomes, which in turn can translate to reduced dengue related hospital stay and mortality.
- The admission and discharge criteria may vary among various clinicians attending patients and may interfere with the results of our findings. However, in our institution patients are routinely discharged on the basis of laboratory findings and clinical conditions.

Introduction

Dengue is among the most important arthropod-borne disease that has rapidly been spread in several regions of the world in recent years. The disease is widespread throughout the tropics, with local variations in risk, influence by rainfall, temperature and unplanned rapid urbanization (1). The spectrum of disease varies from mild self-limiting illness, dengue fever (DF) to more severe and fulminating forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (2). The World Health Organization (WHO) estimates that about 40% of world`s population live in areas having risk of dengue transmission. The surge in dengue has been most marked in Asia, with an estimated 1.8 billion people at risk of dengue (3). Southeast Asia in particular has been large epidemics of the disease in recent years with attendant mortality from dengue viral infection (DVI) (4).

Currently, Malaysia is the leading country in terms of the number of dengue cases reported worldwide (5). Dengue is among top five notifiable diseases in the country (6) and continues to be a formidable public health concern. Malaysia is hyperendemic for dengue and experiencing worst dengue crisis nowadays, while some countries in Southeast Asia including Philippines and Thailand have seen decreases in DVI activity in 2014. Reported dengue cases in Malay Archipelago in 2014 increased dramatically (160%) to 98,128 with 189 deaths, compared to 37,698 with 79 deaths in the previous year (7-9). It is worthwhile to mention that year 2015 painted a scary picture, as highest number of dengue cases (N=111,285) along with 301 deaths were ever recorded in the history of the country (Figure 1) (10). More recently, 94 dengue-related deaths have been reported to WHO until April 2016, compared to a total of 120 deaths during the same reporting period in 2015 (3).

Figure 1

The Case Fatality Rate (CFR) due to dengue varies among countries, but can be as high as 10 – 15% in some and <1% in others (11, 12). The recent epidemiology of dengue in Malaysia has imposed substantial economic and disease burden on patients and health care system with increased hospital stay, high morbidity and attendant mortality. Despite exponential increase in dengue-related deaths in Malaysia, data describing clinico-laboratory characteristics and factors associated with fatal cases are scarce. Efforts made by Sam *et al.* lack statistical comparison between fatal and non-fatal cases and are limited to DHF cases (13). These findings necessitate the urgent need of studies to differentiate clinico-laboratory characteristics among fatal and non-fatal dengue cases. On the other hand, we previously reported that DVI requires longer hospital stay irrespective of the disease severity resulting in significant burden in terms of health service costs. This is of particular importance in resource limited setting, especially in dengue endemic regions (14). Early identification of risk factors associated with prolonged hospital stay and mortality can help physicians to primacies the management of high-risk dengue patients. These factors could also be utilized in formulating predictive score to identify severely ill patients during future outbreaks and to prioritize care that may translate into reduced morbidity and mortality. In this context, a retrospective case series was intended to investigate clinico-laboratory characteristics associated to the prolonged hospitalization (>3 days) and mortality among dengue patients admitted to the tertiary care hospital.

Materials and Methods

Ethical Approval

The study was approved by Human Resource Ethics Committee (JEPeM) of HUSM (USM/JEPeM/14080278). All data was analyzed anonymously and hence, informed consent was not required. The patients were identified from a central computerized record with their registration number (RN). Data of the cases were retrieved and specific numeral codes were given to each case before data analysis. Identity of all patients was not disclosed in current study.

Study location and Population

Current study was conducted in Hospital University Sains Malaysia (HUSM), tertiary level teaching hospital with 950 beds that serves an estimated 1.4 to 1.8 million inhabitants of Kelantan. Kelantan is an agrarian state located in the north-east of Peninsular Malaysia and among top five dengue hotspots in the country where the dengue cases are substantially rising every year. Malays are major (95%) ethnic group in Kelantan while Chinese constitutes merely 4% of state population. The hospital also serves as referral centers for nearby states to treat severe and complicated dengue cases and has reliable medical records (14, 15).

All the dengue patients admitted to the hospital during the period of six years (January 2008 to December 2013) were included into the study. Patients having age ≥ 12 years admitted with primary and confirmed diagnosis of DVI, irrespective of severity, were identified by registration number (RN) using hospital record management system. The process of patient's selection and identification along with inclusion and exclusion criteria are described in Figure 2.

Figure 2

Diagnosis of Dengue

Suspected dengue cases were confirmed by laboratory criteria that were further subjected to clinical case definition of DVI. Suspected dengue infection was defined as the presence of fever and any two of the following symptoms: myalgia, headache, arthralgia, skin rash, retro-orbital pain, hemorrhagic manifestation (s), or leucopenia (white blood cell [WBC] count of $<4\times10^{9}$ L−1). Suspected cases were confirmed by using at least one of the following criteria: (i) positive reverse transcriptase polymerase chain reaction (RT-PCR) result, (ii) presence of dengue immunoglobulin M and G antibodies in acute phase serum by enzyme linked immunosorbent assay [Pan Bio Dengue IgM ELISA, Dengue IgM Dot Enzyme Immunoassay, SD Dengue IgM and IgG capture ELISA Kits; Standard Diagnostics, Korea], and (iii) at least 4-fold increase of dengue-specific hemagglutination inhibition titers in convalescent serum when compared with acute phase serum. The serum samples were also tested for dengue-specific NS1 [pan-E Early dengue ELISA kit by Panbio, Australia and Platelia dengue NS1Ag assay by Bio-Rad Laboratories, USA). Only confirmed dengue cases were included in analysis. Primary dengue infection was distinguished from secondary infection by using IgM-IgG ratio where dengue infection was defined as primary if ratio ≥ 1.8 and as secondary if < 1.8 or if there was a 4-fold increase of HAI and the titers were ≤1:1280 and ≥1:2560, respectively (14). Serologically confirmed dengue patients were subjected to clinical case definition and disease severity was classified into DF, DHF and DSS, according to the WHO criteria (16).

Data Collection and Management

All the required data were collected on structured data collection form, approved by hospital ethical committee. After identification of patients with confirmed dengue, numeral codes were given to patients and these codes were used as identifier during data analysis. Usually

patients with dengue infection have hospital stay between 3 or 4 days (14, 17), therefore we used greater than 3 days as cutoff point for prolonged hospitalization (Median hospital stay in present study was 3 days). Patients having hospital stay ≤3 days were compared with those staying >3 days in order to identify possible predictors of increased hospitalization. Similarly, we stratified all patients into fatal and non-fatal cases and their clinical and laboratory characteristics were compared. Patient`s demographics and clinical presentations were recorded on day of admission while laboratory findings were recorded for each day of hospitalization until discharge or death, whichever occurred first.

Definitions

For the purpose of current study, terms used were defined as follow:

Hospital stay is defined by ≥1 day bed occupancy in hospital; mortality means death within 14 days after admission; hypokalemia (K < 3.5 mmol/L); hyponatremia (Na < 135 mmol/L); oliguria (UO < 400 ml/day after 24 hours of appropriate hydration); hypotension (blood pressure < 110/70 mmHg); elevated transaminases (elevation of liver enzymes such as aspartate aminotransferase [AST] and/or alanine aminotransferase [ALT] >2 times the normal value); prolonged prothrombin time (PT > 15 seconds); prolonged activated partial thromboplastin time (aPTT > 35 seconds); urinary sedimentations (presence of glycosuria, hematuria, proteinuria, leucocytouria, urine pus, urine epithelial cells); anemia (Hb < 12 g/dL); dengue viral infection (dengue fever, dengue hemorrhagic fever, dengue shock syndrome); Acute kidney injury (AKI) (Acute Kidney Injury Network (AKIN) of classification); stages of AKI based upon serum creatinine values (AKIN-I, AKIN-II, AKIN-III); severe dengue (DHF, DSS); multiple organ dysfunction (dysfunction of ≥2 organs); hepatic dysfunction (elevation of liver enzymes); and thrombocytopenia (platelets count < 100×10^9 cells). Reference values of laboratory parameters in

current study are according to the hospital pathology lab included AST (5-34 IU/L); ALT (10-35 IU/L); ALP (♂: 53-168 ♀: 42-98 IU/L); Hematocrit (♂:37.5-49.8 ♀: 31.8-42.4); Platelets (158-410×10^9/L); WBCs (♂:3.8-9.7 ♀: 3.4-10.1); PT (12-13 seconds); aPTT (30-50 seconds).

Statistical analysis

All the patients were divided into two groups based upon presence or absence of outcomes (mortality, prolonged hospital stay). For quantitative variables, measures of central tendency and dispersion were calculated. Qualitative variables are presented as frequencies and proportions for which frequency was served as numerator and total number of patients (n=667) was served as denominator. Relevant denominator was stated before proportion, where it varied. Comparison of categorical variables between two groups was done by using Chi-Square test (if at least 80 percent of cells have expected frequencies of 5 or more) or Fisher`s Exact test (if less than 80 percent of cells have expected frequencies of 5 or more). Comparison of continuous variables was done by an independent Student's t-test. Logistic regression was used to estimate the associations between prolonged hospital stay, as the response variables, and potential predictors. These potential predictor variables were chosen on the basis of statistical significance and their biological plausibility with the outcomes. Co-linearity diagnostics was performed on variables selected for regression analysis. The strength of association was evaluated using an odds ratio (OR) and a 95% confidence interval (CI). The variables with univariate *p*-value less than 0.25 were subjected to multivariate analysis (18). The use of univariate *p*-values <0.25 has advantage of tending to include more variables in multivariate analysis while traditional levels of *p*-value such as 0.05 can fail in identifying variables known to be important (19). Calibration of final multivariate logistic model (model fit) was assessed by Hosmer-Lameshow test. The two-sided statistical significance level, p-value, was set at 0.05 for all inferential analyses in this

study. Data were compiled and analyzed using Statistical Package for Social Sciences program version 20 (SPSS: Inc. Chicago. Il. USA).

Results

Out of the total dengue cases admitted to the hospital, 667 patients with mean age 30.8 ± 16.1 years were included in analysis (Figure 2). According to WHO criteria, DF was diagnosed in 627 (88.1%) patients while DHF (DHF grade I & II) and DSS (DHF grade III & IV) were observed in 74 (11.1%) and 5 (0.8%) cases, respectively. Most of the studied participants were ethnic Malay (90.4%) followed by Chinese (7.9%) and Indians (1.4). The most common non-hemorrhagic manifestations at hospital admission were fever (97%), headache (58.9%), retro-orbital pain (25%), myalgia (70.4%), arthralgia (56.5%), nausea (31.2%), vomiting (54.2%), abdominal pain (44.7%), cough (18.5%), chills (31.5%), anorexia (27%), malaise (7.3%), lethargy (27.4%), palpitation (2%), flushing (19%), dehydration (11%), ascites (2.7%) and pleural effusion (3.2%). On the other hand, petechia (11.2%), hematemesis (1.9%), purpura ecchymosis (7.4%), epistaxis (4.9%), hematuria (2.5%) and gum bleeding (9.4%) were common hemorrhagic presentations among dengue patients.

The mean length of hospital stay (LOS) was 4.88 ± 2.74 days (median 3, IQR 3, range: 1-35 days). Prolonged hospitalization (> 3 days) was seen in 49.2% (n = 328/7667) patients while length of stay (LOS) was ≤ 3 days among 50.8% (n = 339/667) studied participants. Clinical characteristics of patients with and without prolonged LOS were compared (Table 1). Patients having DHF, hypertension (HTN), elevated ALP, multiple organ dysfunctions (MODs), prolonged PT and aPTT were signicantly associated with prolonged hospitalization. Although, other factors such as secondary infection, comorbidities, acute kidney injury (AKI), elevated AST and hematocrit > 20% were more profound among patients with prolonged hospitalization

but their statistical association was insignificant (P>0.05). It was interesting to note that male gender and patients with DF were more likely to stay ≤ 3 days in the current study (Table 1).

Table 1

To determine the factors independently associated with prolonged hospitalization, we developed a series of logistic regression analyses, which are shown in Table 2. Out of eight readily available clinical parameters that had p-values <0.25 in the univariate analysis, four factors (DHF, elevated ALP, increased PT/aPTT, MODs) were found to be independently associated with prolonged length of stay (LOS). Although, presence of hypertension and thrombocytopenia were found as risk factors of prolonged LOS in unadjusted analysis but neither of these showed statistically significant results in multivariate model. Though, thrombocytopenia and AKI were insignificant variables in Table 1 but included in logistic regression due to their hypothetical and clinical association with prolonged hospitalization. Receiver operating characteristics (ROC) curve analysis with AUC as 0.812 demonstrated that logistic model has good ability to predict prolonged hospitalization among dengue patients (Figure 3).

Table 2

Figure 3

Evaluation of Dengue related Fatal cases

The overall dengue case fatality rate (CFR) was 1.1% and all eight fatal cases were attributed to the dengue infection. Of the dengue deaths, 4 (50%) were male, 6 (75%) were Malay and two (25%) were Chinese. The mean age was 48.8 ± 25.6 years (range: 13-78) and most of the fatal

cases (n=6/8, 75%) were rural residents. DF was present in 6/8 (75%) patients while DHF was recorded in 2/8 (25%) cases. Four patients (50%) had no pre-existing comorbidities and remaining four patients (50%) were having at least two comorbid conditions (Table 3).

The mean duration of hospital stay among death cases was 5.6 ± 3.2 days (median: 5, IQR: 9, range 3-12 days) and all of them were admitted ≥5th day of onset of illness. The number of fatal cases in the present study was very small that precluded us to perform logistic regression analysis. However, results of Chi-square analysis demonstrated significantly higher proportion of age > 40 years, secondary infection, comorbidities (DM, IHD), AKI, prolonged PT, MODs, hemoconcentration, rhabdomyolosis and respiratory failure among death cases as compared to patients who survived (Table 3).

Table 3

The main presenting complaints in all fatal cases are shown in Table 4. Fever, nausea, vomiting and abdominal pain were presented in all death cases while retro-orbital pain and dysuria were present in 6/8 (75%) patients.

Table 4

Table 5 showed individual data points of all eight patients who died. All of them were brought alive to the hospital and succumbed to the infection within 3-12 days of admission. These patients were admitted on or after day 5 of illness and had rapid deterioration of their clinical features resulting admission to the intensive care unit (ICU). Dengue infection was confirmed in all patients by dengue serological tests. Three patients had clinical history relating to dengue fever. About one third patients died within 72 hours of hospital admission. On

admission, all the cases were febrile (mean 38.9 ^{o}C) and about 50% patients had pulse rate >100 beats per minute (BPM). We observed multifactorial causes of death in the current study including dengue infection complicated with multiple organ dysfunctions (100%), acute kidney injury (100%), shock (25%), acute respiratory distress syndrome (25%), disseminated intravascular coagulation (12.5%), gastric bleeds (25%) and underlined comorbid conditions (50%). Five died patients (62.5%) had prolonged hospital stay in the current study. Unfortunately, we had no access to postmortem and autopsy data of fatal cases.

Table 5

Discussion

The alarming rise of dengue epidemiology has been highlighted to haunt 40% of world population. In Malaysia, dengue is perceived as a highly contagious health threat with escalating trend of infection. The average number of dengue cases and death tolls had recorded a high surge over the past few years leading to substantial disease burden in terms of cost. Of the estimated, the annual cost for dengue illness (standard errors in parenthesis) in Malaysia is US$42.4 (±4.3) including per capita cost US$4.73 for one thousand population size (n=1000) with disability adjusted life years (DALYs) equivalent to 8,324 (20). As stated earlier, dengue induced disease burden related to cost of care and mortality is of particular importance, especially in developing countries where the dengue is endemic (15). Early identification of dengue patients having high risk of prolonged hospital stay and death may serve as an effective tool to combat the upsurge of disease burden. In this attempt, we evaluated several factors associated with prolonged hospitalization and mortality among dengue patients attending tertiary care hospital.

Approximately half of the studied participants had prolonged hospitalization that carry substantial economic, societal and personal burden. In our recent series, we reported that prolonged hospital stay is not only associated with DHF but also with classical DF (14). The proportion of prolonged hospitalization in DF and DHF was 46.7% (n=275/588) and 67.1% (n=53/79), respectively. These findings are in agreement with previous studies describing association of dengue infection with prolonged hospitalization, irrespective of severity (14, 15, 21). We found statistical association of DHF and prolonged hospital stay in the present study, where individuals with DHF had twice higher odds of longer hospital stay than patients without DHF. The mean hospital stay in present series (4.47 days) is consistent with previous

investigations reporting mean hospitalization between 3.4 to 6.2 days (14, 21-24). Comparative analysis revealed that dengue patients with hypertension, elevated ALP, prolonged PT/aPTT and multiple organ dysfunctions (MODs) were strongly associated (P<0.005) with hospital stay > 3 days (Table 1). These findings are in concordance with the results of one and only study evaluating predictors of longer hospitalization among dengue patients (21).

Many risk factors were identified and evaluated independently in their contribution to prolonged hospitalization (Table 2). Of these, presence of DHF, elevated ALP, prolonged PT/aPTT and MODs were found to be predisposing factors of longer hospital stay. Though, Khalil *et al.* reported old age and AKI as predictors of increased hospital stay but present study did not show any statistical association of these variables with length of stay (21). Age was equally distributed among patients with and without longer hospital stay in our study (Table 1). It might be due to population differences, as Khalil and colleagues included patients aged > 14 years while we included patients with age ≥12 years in our study. Several previous case series have demonstrated the association of elevated SCr or AKI with prolonged hospitalization and death (14, 15, 21, 25, 26). Although, prevalence of AKI in our study was high (14.2%) but was equally distributed (P=0.242) among patients with and without prolonged hospitalization (Table 1). However, subgroup analysis showed that patients with AKI had signicantly (p<0.001) longer duration of hospital stay than patients without AKI. Our findings, in addition to several other studies suggest the monitoring of serum creatinine while managing the dengue patients.

Significant number of patients in our study had coagulopathy attributed to low platelets, deranged PT, aPTT and elevated liver transaminases (Table 1). More than half of the study participants with prolonged PT and aPTT were associated with longer hospitalization (Table 1). Involvement of liver has been well documented during the course of dengue infection (27).

However, only elevated ALP was observed as an independent predictor of longer hospital stay. Moreover, dysfunction of several other organs including brain, heart, muscles, spleen, and gallbladder has recently been emerged as expanded dengue syndrome (EDS) and might be contributed directly to the viral localization in organs or to development of ischemia of various organs as a result of AKI, coagulopathy and old age (16, 28). Dengue patients with multiple organ dysfunctions (MODs) had two times higher risk of longer hospital stay in the present study. Notably, DHF in combination with MODs, hypertension, coagulopathy and elevated ALP might denote seriously sick patients who can potentially have more morbidity in the form of increased hospital stay. Identification of these patients at the earliest and their management with special care would be advantageous in reducing morbidity and hence their bed occupancy in the hospital.

Dengue viral infections are rarely fatal, although fatal infections do occur due to plasma leakage, fluid accumulation, respiratory distress, severe bleeding or MODs (14). Overall mortality in current study was 1.1% that is consistent with previous national (13, 14) and global studies (21, 26, 29). Malaysia experienced large dengue epidemics in the past decade affecting predominantly adults (5). This shift from childhood to adult illness is attributed to lower herd immunity and transmission outside home (5, 14). Though, current study included patients with age > 12 years but we did not observe any mortality among patients aged < 12 years during the study period (data is not shown). Two out of eight died cases belonged to adolescent age group while remaining had advanced age above 40 years (Table 3 & 5). Interestingly, dengue patients with age >40 years were significantly associated with mortality in the current study. The higher risks of dengue related deaths with increasing age might be contributed to decline in physiological functions and underline diseases in aging people. In addition to comorbidities,

older patients would have rehabilitation issues which may complicate admission and extend hospital stay. Dengue patients with age >50 years were reported to be at high risk of hospitalization and mortality in two separate series (30, 31). Prolonged hospitalization as defined in current study was observed in 50% (n=4/8) of fatal cases. This study explicitly illustrates that in addition to high mortality rate, the advanced age is also associated with increased length of hospitalization. These findings are consistent with Taiwanese study suggesting the impact of increasing age with higher fatality and longer hospitalization (32). On the other hand, it can also be assumed that dengue patients with advanced age staying more than 3 days in the hospital should be considered as high risk patients for mortality. However, we recommend large multicentric controlled studies to evaluate this assumption. On the other hand, we found equal distribution of gender among fatal cases (Table 3) and this is in contrast with the findings of Malaysian study reporting preponderance of dengue deaths amongst females (13).

It is interesting to note that all fatal cases had severe AKI and multiple organ dysfunctions (MODs) (Table 3 & 5). These findings are in agreement with the results of Khalil *et al*. who reported AKI in all fatal cases (21). In another case series, Lee *et al.* reported the presence of AKI in 80% patients who died (32). Moreover, AKI as a cause of death has been documented in two separate studies reporting prevalence of AKI in 30% and 14% of fatal cases (13, 29). We also presented similar findings where severe AKI was among documented causes of death (Table 5). This study also found that secondary infection, diabetes mellitus, IHD, prolonged PT, hematocrit >20%, rhabdomyolosis and respiratory failure were associated with dengue-related mortality in the overall study population (Table 3). Current data also suggest that rhabdomyolosis and coagulation disorders are not uncommon intricacies in dengue infection,

implicating that clinicians should be alert to the possible occurrence of these complications when caring dengue patients.

Secondary infection usually occurs in dengue endemic regions and correlates to disease severity (15). Three of the fatal cases in this series had secondary infection while remaining five cases had evidence of primary infection (Table 5). Although, this observation is in contrast to the other reports illustrating occurrence of mortality predominantly in secondary infection (11, 13, 30) but in agreement with the findings of Ong *et al*. where 3 out of 7 death cases had primary infection (29).

Mortality is usually linked to delayed provision of supportive treatment and/or pre-morbid chronic illness (14, 16). All died cases were admitted on day five (range: 5 – 8 days) of illness and most of them had defervescence, followed by rapid deterioration of clinical condition. In our study, the average duration of illness prior to hospitalization among death cases was significantly differ from those who survived (P=0.025). Our observation is consistent with earlier studies done on dengue deaths where late hospitalization was found to be a possible contributing factor to increased risk of mortality (11, 13, 29). These findings suggest that early care-seeking behavior may determine the possibility of receiving and thereby avoiding fatal outcomes.

Besides care-seeking behavior pattern, the presence of comorbidities is of paramount importance among dengue patients. Several reports suggested that worsening of co-morbid conditions, rather than directly from dengue infection could be the reason for death seen especially in adults (33, 34). Half of the death cases in present study had underlying comorbid illness including CKD, DM, HTN and IHD. Patients with diabetes and IHD had higher risks of death, when compared to their survived counterparts (Table 3). Several earlier reports implicated

DM as a possible contributing factor to death (33, 34). Nonetheless, 50% death cases were healthy individuals without any comorbid condition. This observation highlighted that underlying co-morbidities might not be the main factor contributed to the dengue mortality in our cohort.

Good early predictors of mortality are presently lacking. Several reports emphasized the importance of individual warning signs as tool to recognize patients having high risk of severe illness and death (11, 34, 35). All the fatal cases in our study had at least one warning sign (Table 4) and these findings are consistent with Cuban study reporting presence of warning signs in all twelve fatalities (11). Contrary to these findings, Ong *et al.* reported presence of warning signs in 50% of death cases (29). Hepatomegaly commonly occurs in severe dengue (36) and was observed in two patients in our series. Hepatospleenomegaly usually associated with macrophage activation syndrome (MAS) and was present in only one patient, suggesting its association with dengue infection. Such association has also been previously reported in the literature (37). These observations underscore that attention should be paid to uncommon presentations because they may often related to poor prognosis and high mortality. The causes of death in current study are consistent with those reported in other series on dengue fatal cases (11, 13, 38).

This retrospective study identified several factors associated with prolonged hospital stay and mortality among dengue patients attending tertiary care hospital. These factors should be identified at the earliest and treated preferably in a special care setup. Use of advanced therapies (renal replacement, ventilator support) along with inotropes or antibiotics (for hemodynamic disturbances and sepsis), can potentially improve patient`s outcomes, which in turn can translate to reduced dengue-related hospital stay and mortality.

Study Limitations

However several shortcomings of the present study should be considered. This study is a single-center study with retrospective analysis and the results may therefore not necessarily be generalized to the other populations. Additionally, study depends on thoroughness of clinician`s documentation so disease severity and clinical outcomes of included patients may be biased due to lack of standardized management protocol for dengue. The admission and discharge criteria may vary among various clinicians attending patients and may interfere with the results of our findings. The small number of fatal cases in our study may make statistical power quite small for identification of factors associated with dengue-related mortality. However, four case series with limited number of fatal cases (n=7, n=5, n=19, n=18) evaluating factors of mortality support the findings of present study (29, 32 , 38, 39). A case control study with much larger sample size is needed to determine whether clinico-laboratory findings seen in our series are exclusive to fatal cases of dengue or seen equally in non-fatal cases. Moreover, study population included adults and hence results cannot be generalized to pediatric patients. Unfortunately, autopsy and postmortem data of died patients were not available. Last but not least, WHO 1997 criteria of dengue classification were used in the current study because similar criteria are being used in Malaysia. A recent investigation has also verified diagnosis of dengue using WHO 1997 classification in Malaysia (40). However, health authorities included WHO 2009 criteria in new guidelines on dengue infection that issued in November, 2016.

Nevertheless, the strength in this study lies in our attempt to include large heterogeneous cohort of dengue population. Present study improves awareness of factors associated with prolonged hospitalization and death among dengue patients. It also highlights the need for more studies and for strategized management protocol in order to reduce the disease

burden. Moreover, this is only largest study conducted in Malaysia describing factors relating to prolonged hospitalization. These observations along with our previous case series (14, 15, 18, 25, 28) prove to be a valuable addition in dengue literature and warrants further investigations.

Conclusion

Our case series demonstrates high proportion of dengue patients with prolonged hospital stay. Patients with severe disease (DHF) along with elevated ALP levels and deranged PT/aPTT had higher likelihood to stay more than 3 days in the hospital. Our observations exemplified that fatal dengue infection does occur in adults and in primary infection, irrespective to gender. Factors including advance age, secondary infection, comorbidities, prolonged PT, hemoconcentration and rhabdomyolosis were found to be statistically associated with mortality. Early identification of dengue patients with these factors will have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units. Attention to these high risk patients may have a positive impact on both patients and health care system by reducing dengue related bed occupancy and mortality.

Recommendation

For clinical practice, doctors treating dengue especially those working in the front-line should be made aware of the factors significantly associated with longer hospital stay and dengue mortality. With proper identification of these factors, time and resources can be focused on those at highest risk.

Conflict of interest: Authors declare no conflict of interest.

Funding: There is no funding for this project.

Author`s contribution: THM, YHK and ASA provided substantial contributions to the conception or design of the work. THM and AS were involved in the acquisition analysis and interpretation of data. THM and AHK were responsible for drafting the work or revising it critically for important intellectual content. AHK and ASA gave final approval of the version to be published. All the authors substantially contributed to the manuscript and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Sharing: All the relevant data are within manuscript.

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Figure Legends

Figure 1: Incidence of dengue cases and deaths in Malaysia (Ministary of Health Malaysia, epidemiological data of WHO and MOH may slightly vary due to the differences in reporting criteria or lack of reporting)

Figure 2: Methodological Flow of Study

Figure 3: ROC curve analysis of multivariate logistic model predicting prolonged hospitalization among dengue patients (AUC: area under the curve, CI: confidence interval)

Table 1: Comparison of clinical characteristics (on-admission) of dengue patients according to the presence or absence of prolonged hospital stay (>3 days)

Variables	Total cohort N=667	LOS in hospital		P values
		≤ 3 days (N=339)	>3 days (N=328)	
Age (years), mean±SD	30.8 ± 16.1	30.7 ± 15.8	30.7 ± 16.5	0.305
Age >40 yrs, n (%)	167 (25%)	84 (24.5%)	83 (25.3%)	0.875
Age >60 yrs, n (%)	35 (5.2%)	17 (5.1%)	18 (5.5%)	0.784
Male gender, n (%)	378 (56.7%)	189 (55.8%)	139 (42.4%)	0.626
Secondary infection, n (%)	73 (10.9%)	33 (9.7%)	40 (12.2%)	0.309
Dengue severity, n (%)				
DF, n (%)	588 (88%)	313 (92.3%)	275 (83.8%)	0.001
DHF[§], n (%)	79 (10.5%)	26 (7.7%)	53 (16.2%)	**0.001**
Warning signs, n (%)	271 (40.6%)	141 (41.6%)	130 (39.6%)	0.607
Comorbidities, n (%)				
DM, n (%)	36 (5.4%)	14 (4.1%)	22 (6.7%)	0.141
HTN, n (%)	35 (5.2%)	12 (3.5%)	23 (7%)	**0.044**
CKD, n (%)	33 (4.9%)	15 (4.4%)	18 (5.5%)	0.522
IHD, n (%)	25 (3.7%)	9 (2.7%)	16 (4.9%)	0.131
Comorbidities ≥2, n (%)	36 (5.4%)	13 (3.8%)	23 (7%)	0.069
AKI, n (%)	95 (14.2%)	43 (12.7%)	52 (15.9%)	0.242
Scr ≥ 2mg/dL, n (%)	29 (4.1%)	10 (2.9%)	19 (5.8%)	0.048
Elevated ALT, n (%)	362 (54.3%)	192 (56.6%)	170 (51.8%)	0.197
Elevated AST, n (%)	447 (67%)	226 (66.7%)	221 (67.4%)	0.636
Elevated ALP, n (%)	133 (19.9%)	44 (13%)	89 (27.1%)	**<0.001**
Prolonged PT, n (%)	224 (33.3%)	80 (23.6%)	144 (43.9%)	**<0.001**
Prolonged aPTT, n (%)	159 (23.8%)	52 (15.3%)	107 (32.6%)	**<0.001**
Thrombocytopenia, n (%)	395 (59.2%)	191 (56.3%)	204 (62.2%)	0.134
MODs, n (%)	117 (17.5%)	44 (13%)	72 (22.3%)	**0.002**
Hematocrit > 20 %, n (%)	73 (10.9%)	38 (11.2%)	35 (10.7%)	0.824

[§]DHF includes all four grades

P values were calculate between patients with and without prolonged hospital stay

Abbreviations: LOS: length of stay, DF: dengue fever, DHF, dengue hemorrhagic fever, DSS: dengue shock syndrome, DM: diabetes mellitus, HTN: hypertension, CKD: chronic kidney disease, IHD: ischemic heart disease, AKI: acute kidney injury, Scr: serum creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, MODs: multiple organ dysfunction

Table 2: Univariate and Multivariate analysis to evaluate determinants (risk factors) of prolonged length of hospital stay (LOS)

Variables*	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI	*P*-value	OR	95% CI
DHF	0.001	2.3	1.4 to 3.8	**0.009**	2.3	1.2 to 4.3
Hypertension	0.048	2.1	1.1 to 4.2	0.688	1.2	0.5 to 2.1
Elevated ALP	<0.001	2.5	1.7 to 3.7	**<0.001**	2.3	1.5 to 3.5
Prolonged PT	<0.001	2.6	1.9 to 3.6	**0.009**	1.7	1.1 to 2.5
Prolonged aPTT	<0.001	2.7	1.8 to 3.9	**0.005**	1.9	1.2 to 2.9
Thrombocytopenia	0.135	1.3	0.9 to 1.7	0.197	1.3	0.9 to 1.8
AKI	0.242	1.3	0.8 to 2.0	0.174	0.7	0.3 to 1.2
MODs	0.002	1.9	1.3 to 2.9	**0.013**	2.1	1.2 to 3.7

*Variables lack multi-collinearity and had variance inflation value (VIF) less than 2
Hosmer-Lameshow Test Statistics: Chi-square: 7.474, degree of freedom: 6, p value = 0.279
Abbreviations: DHF: dengue hemorrhagic fever, HTN: hypertension, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, AKI: acute kidney injury, MODs: multiple organ dysfunction

Table 3: Comparison of clinical characteristics (on-admission) of fatal and non-fatal dengue cases

Variables	Total cohort N=667	Non-Fatal Cases N = 659	Fatal cases N = 8	P* values
Age (years), mean±SD	30.7 ± 16.1	30.5 ± 1597	48.8 ± 25.6	0.083
Age >40 yrs, n (%)	167 (25%)	161 (24.4%)	6 (75%)	**0.004**
Age>60 yrs, n (%)	35 (5.2%)	33 (5.0%)	2 (25%)	0.061
Male gender, n (%)	378 (56.7%)	374 (56.8%)	4 (50%)	0.732
Secondary infection, n (%)	73 (10.9%)	70 (10.6%)	3 (37.5%)	**0.047**
Dengue severity, n (%)				
DF, n (%)	588 (88%)	582 (88.3%)	6 (75%)	0.243
DHF[§], n (%)	79 (10.5%)	77 (11.7%)	2 (25%)	0.243
Comorbidities, n (%)				
DM, n (%)	36 (5.4%)	33 (5.0%)	3 (37.5%)	**0.007**
HTN, n (%)	35 (5.2%)	33 (5.0%)	2 (25%)	0.061
CKD, n (%)	33 (4.9%)	31 (4.7%)	2 (25%)	0.055
IHD, n (%)	25 (3.7%)	23 (3.5%)	2 (25%)	**0.033**
Comorbidities ≥2, n (%)	36 (5.4%)	33 (5.0%)	4 (50%)	**0.007**
AKI, n (%)	95 (14.2%)	87 (13.2%)	8 (100%)	**<0.001**
Scr ≥ 2mg/dL, n (%)	29 (4.1%)	21 (3.2%)	8 (100%)	**<0.001**
Elevated ALT, n (%)	362 (54.3%)	360 (54.6%)	2 (25%)	0.151
Elevated AST, n (%)	447 (67%)	443 (67.2%)	4 (50%)	0.112
Elevated ALP, n (%)	133 (19.9%)	133 (20.2%)	0	-
Prolonged PT, n (%)	224 (33.3%)	218 (33.1%)	6 (75%)	**0.022**
Prolonged aPTT, n (%)	159 (23.8%)	157 (23.8%)	2 (25%)	1.000
Thrombocytopenia, n (%)	395 (59.2%)	393 (59.6%)	2 (50%)	0.072
MODs, n (%)	117 (17.5%)	109 (16.5%)	8 (100%)	**<0.001**
Hematocrit > 20 %, n (%)	73 (10.9%)	68 (10.3%)	5 (62.5%)	**0.001**
Rhabdomyolosis, n (%)	49 (6.9%)	42 (6.4%)	7 (87.5%)	**<0.001**
Respiratory Failure, n (%)	11 (1.5%)	9 (1.4%)	2 (25%)	**0.007**

[§]DHF refers to all grades including 5 cases of DSS
*P values were calculate between patients with and without prolonged hospital stay
LOS: length of stay, DF: dengue fever, DHF, dengue hemorrhagic fever, DSS: dengue shock syndrome, DM: diabetes mellitus, HTN: hypertension, CKD: chronic kidney disease, IHD: ischemic heart disease, AKI: acute kidney injury, Scr: serum creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, PT: prothrombin time, aPTT: activated partial thromboplastin time, MODs: multiple organ dysfunction

Table 4: Clinical Features (presenting complaints) of Dengue Fatal Cases (n=8)

Sign and Symptoms	P1	P2	P3	P4	P5	P6	P7	P8	n (%)
Fever	×	×	×	×	×	×	×	×	8 (100%)
Nausea	×	×	×	×	×	×	×	×	8 (100%)
Vomiting	×	×	×	×	×	×	×	×	8 (100%)
Abdominal pain	×	×	×	×	×	×	×	×	8 (100%)
Retro-orbital pain	×	×	×	×		×	×		6 (75%)
Dysuria	×	×		×	×		×	×	6 (75%)
Chills	×		×	×		×			4 (50%)
Headache		×	×			×	×		4 (50%)
Myalgia		×				×	×	×	4 (50%)
Diarrhea	×	×		×			×		4 (50%)
Malaise		×	×			×	×		4 (50%)
Lethargy		×			×		×	×	4 (50%)
Restlessness					×			×	2 (25%)
Jaundice			×		×	×		×	4 (50%)
Rigors		×	×			×	×		4 (50%)
Skin rash	×	×		×			×		4 (50%)
Pleural effusion	×						×		2 (50%)
Shortness of breath	×						×		2 (25%)
Dizziness				×				×	2 (25%)
Anorexia		×					×		2 (25%)
Confusion		×					×		2 (25%)
Asthenia					×			×	2 (25%)
Petechia/ Purpura	×	×							2 (25%)
Gum bleeding	×	×							2 (25%)
Spleenomegaly					×		×		2 (25%)
Hepatomegaly	×						×		2 (25%)
Conjunctivitis			×				×		2 (25%)
Edema	×			×					2 (25%)
Ascites	×		×						2 (25%)
Anascara	×			×					2 (25%)

All the signs and symptoms listed were presented at hospital admission
P1 to P8 demonstrated patients numbers from 1 to 8

Table 5: Demographics, co-morbidities, clinical features and causes of death of fatal dengue patients seen at HUSM between 2008 to 2013

Patients	Age (years) /gender	Duration of illness prior to admission (day)	Secondary infection	Comorbidities	Dengue diagnosis	Duration of hospital stay/duration of fever	Complications/ causes of death*
P1	14 / M	6	Yes	Nil	DHF	4 / 2	DHF complicated with MODs, severe AKI, rhabdomyolosis, respiratory failure, bleeding tendencies, ARDS
P2	78 / F	7	No	IHD, HTN, newly diagnosed OP	DF	6 / 4	Septic shock, probable leptospirosis, DF complicated with MODs, severe AKI, septicemia, rhabdomyolosis, bleeding tendencies, IHD, HTN
P3	43 / F	8	No	Nil	DF	8 / 2	Severe AKI, DF complicated with MODs, rhabdomyolosis, DIC, gastric disturbances, hypotension
P4	13 / M	5	No	Nil	DF	3 / 2	DF complicated with MODs, severe AKI, dehydration secondary to dengue fever, rhabdomyolosis, respiratory failure, ARDS
P5	59 / M	6	No	CKD, DM	DF	3 / 2	Acute on chronic renal failure, severe AKI, DF complicated with MODs, rhabdomyolosis, DM, CKD
P6	44 / F	5	No	Nil	DF	12 /2	Severe AKI , DF complicated with MODs, DIC, dehydration secondary to dengue fever, gastric disturbances, hypotension
P7	77 / F	6	Yes	IHD, HTN, DM	DHF	7 / 4	Septic shock, DHF complicated with MODs, severe AKI, septicemia, rhabdomyolosis, acute myocardial infraction, IHD, HTN, respiratory failure, ARDS
P8	60 / M	5	Yes	DM, CKD	DF	3 / 2	Renal complications, acute on chronic renal failure, DF complicated with MODs, severe AKI, ketoacidosis, rhabdomyolosis, DM, CKD
P1 to P8: number of patients from 1 to 8, F: female, M: male							

	Cases	Deaths
1995	6534	28
1996	14255	32
1997	19429	52
1998	27381	82
1999	101146	37
2000	7103	45
2001	16368	50
2002	32767	99
2003	31545	72
2004	33895	102
2005	39654	107
2006	38556	92
2007	48846	98
2008	49335	112
2009	41486	88
2010	46171	134
2011	19884	36
2012	21900	35
2013	43346	92
2014	108698	215
2015	120836	336

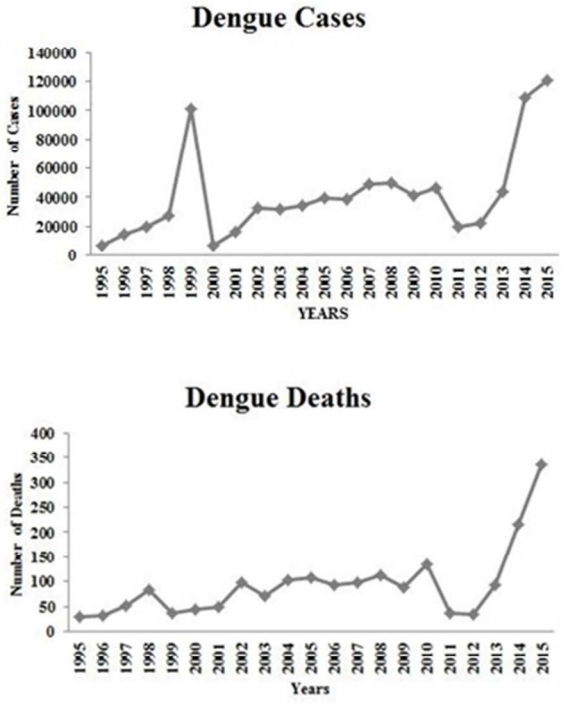


Figure 1: Incidence of dengue cases and deaths in Malaysia (Ministary of Health Malaysia, epidemiological data of WHO and MOH may slightly vary due to the differences in reporting criteria or lack of reporting)

55x44mm (300 x 300 DPI)

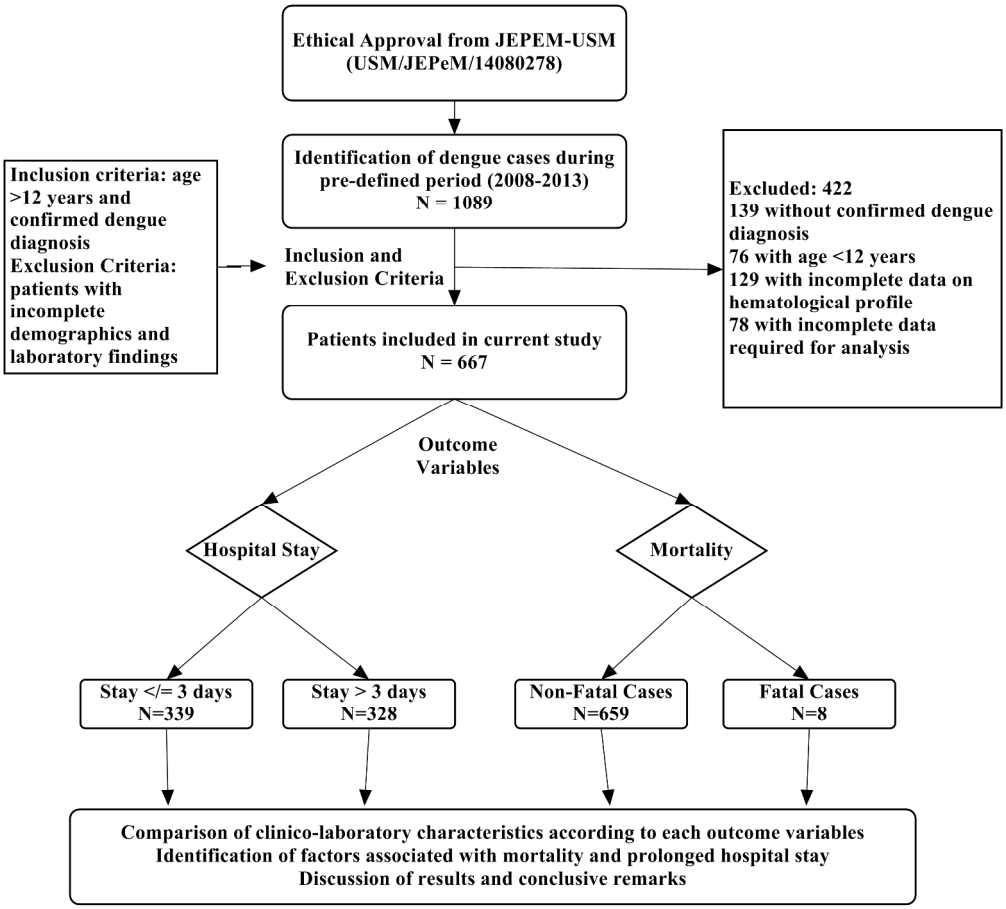
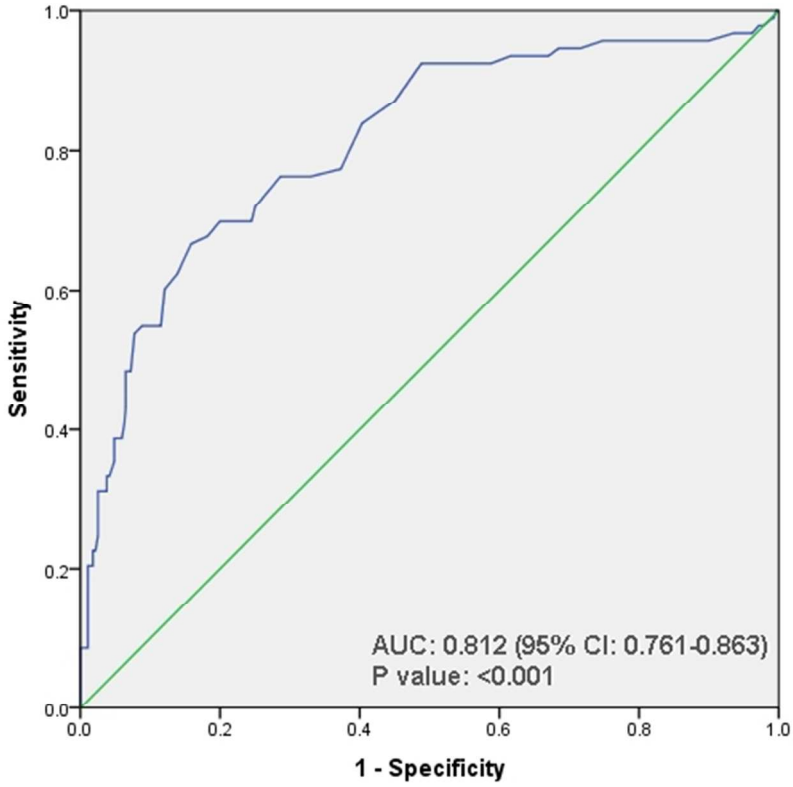


Figure 2: Methodological Flow of Study

285x257mm (300 x 300 DPI)



ROC curve analysis of multivariate logistic model predicting prolonged hospitalization among dengue patients (AUC: area under the curve, CI: confidence interval)

53x42mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	9, 10
		(*c*) Explain how missing data were addressed	9, 10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	9, 10

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9, 10
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	13
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14
		(*b*) Report category boundaries when continuous variables were categorized	13, 14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13, 14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13, 14
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-23
Generalisability	21	Discuss the generalisability (external validity) of the study results	22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	n/a

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.