ORIGINAL ARTICLE

Risk Factors Associated with Development of Dengue Haemorrhagic Fever or Dengue Shock Syndrome in Adults in Hospital Tengku Ampuan Afzan Kuantan

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SUMMARY

A retrospective study was conducted to investigate 183 serologically-confirmed cases of dengue fever (DF) admitted from October 2004 to March 2005 in a large hospital in Pahang. Clinical and laboratory features, progress and outcome of these patients were analysed in order to identify risk factors associated with development of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Individually, we found that older patients, secondary dengue infection, high baseline haematocrit levels, low platelet levels and prolonged activated partial thromboplastin time (APTT) ratio were significant associations with bleeding tendencies. Of these risk factors, haematocrit and APTT ratio were two independent significant risk factors on multivariate analysis. Older patients with primary infection and younger patients with secondary infection had significant bleeding tendencies. We also verified the validity of the haematocrit levels suggested as cut-off levels for plasma leakage for the Malaysian population by Malaysian Clinical Practice Guidelines for Dengue Infection in Adults (2003).

KEY WORDS:
Dengue Haemorrhagic Fever, Dengue Shock Syndrome, Risk factors, Predictor, Haematocrit

INTRODUCTION

Dengue fever is an endemic infective disease in Malaysia. It is an arboviral infection caused by the family *Flaviviridae*. Malaysia reported its first case of DF in 1902 and DHF in 1962. The first major outbreak of dengue haemorrhagic fever (DHF) in Malaysia occurred in 1973, and the country experienced a large epidemic with 3005 notified cases with 35 deaths in 1982[1]. The year 2005 witnessed the highest number of dengue infection in Malaysia with a significant rise compared to a year before. There was a total of 27,659 cases reported in 2005 (till 24th September) with 70 deaths compared to 21,786 cases reported in 2004, with 68 deaths[2]. Pahang had the eighth highest number of dengue cases reported in 2005 (till September 2004) after Selangor, Wilayah Persekutuan Kuala Lumpur, Penang, Johor, Perak, Kedah and Sabah. During that period, there were 1159 cases of dengue infections reported from Pahang compared to 412 cases in 2004[2].

This study aims to determine risk factors associated with DHF/DSS in order to help physicians prioritise the management of high-risk dengue patients. Efforts made by previous workers in the same areas include studies by Narayanan M *et al* (2002)[3], Pancharoen C *et al* (2002)[4], Shivbalan S *et al* (2004)[5], Wichmann O et al (2004)[6] and Diaz-Quijano FA *et al* (2005)[7]. Most of these studies used univariate analysis to identify the risk factors. Shibalan *et al*[5] used multivariate analysis and found platelet of <50,000/mm^3, haemoconcentration and elevated alanine aminotransferase (ALT) of more than three times that of normal value were risk factors for DHF.

MATERIALS AND METHODS

We reviewed all dengue cases admitted to adult medical wards from October 2004 to March 2005 in Hospital Tengku Ampuan Afzan (HTAA), Kuantan, Pahang. Cases with the discharge diagnosis of either confirmed or suspected DF were identified from the hospital database. Data regarding clinical presentations, laboratory investigations and outcome were analysed.

Diagnosis of dengue infection is based on World Health Organization's (WHO) criteria[8] whereby a positive serology and presence of classical symptoms are essential to make a confirmed diagnosis. Serology diagnosis of dengue infection is based on either in-house Enzyme Immunoassay (EIA) or rapid commercial qualitative immuno-chromatographic tests manufactured by Panbio (Brisbane, Australia). The kits used include Dengue Duo Immunoglobulin M (IgM) and Immunoglobulin G (IgG) Rapid Strip Test (Cat No. R-DEN02D), which detects both immunoglobulins on one strip, IgM Capture ELISA (Cat No. E-DEN01M) and IgG Capture ELISA (Cat No. E-DEN02G), which detect IgM and IgG separately. Positive serology is defined as presence of either positive IgM or IgG or both. Equivocal results were excluded if repeat serology was not available. Primary infection is defined as presence of IgM with negative IgG. Secondary infection is defined as positive IgG regardless of IgM result as defined by Vaughn et al (Thailand, Vietnam)[9,10], Chew *et al* (Singapore)[11] and Lam *et al* (Malaysia)[12]. Patients who had only IgM performed without IgG were not assigned to either types of infection.

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"Confirmed Dengue" refers to patients who fulfilled the WHO criteria of acute dengue infection. These cases were included into the analysis of risk factors for DHF/DSS. "Other Diagnosis" refers to those with negative serology and/or well-established alternative clinical diagnosis. "Indeterminate" refers to those patients with inconclusive serology and absence of well-established alternative clinical diagnosis.

"Confirmed Dengue" cases were further classified into two groups, namely "Classical DF" and "DHF/DSS". Definitions of "Classical DF", "DHF" and "DSS" are based on WHO definitions[8].

Statistical Analysis

The data was analyzed using SPSS version 13 (Chicago, Illinois). Univariate analysis was done for all variables. Independent sample t-Test or the equivalent non-parametric Mann-Whitney U Test was used for continuous parameters depending on their distribution. Categorical variables were tested using chi-square test. Odds ratio (OR) was calculated to measure the degree of association. Significance was taken at 0.05.

RESULTS

A total of 358 cases were identified to have discharge diagnosis of DF/DHF/DSS or 'Dengue Suspect'. Out of these 358 cases, 277 (77%) case notes were found and analyzed. Diagnosis was reviewed for these 277 cases and 183 (66%) cases fulfilled the criteria for DF/DHF/DSS (Confirmed Dengue). Eighty one (29%) cases were identified to be "Indeterminate" and 13 (5%) cases were found to be "Other Diagnosis". The breakdowns for "Confirmed Dengue" cases are as shown in Figure 1.

From the total of 183 patients with confirmed dengue, there was almost equal gender distribution with female patients comprising 52.4%. The median age of the subjects was 27 years old and 60.0% of the subjects were less than 30 years old. There were 148 (80.8%) Malays.

Demographic data between the groups with classical DF and DHF/DSS, showed that patients aged 30 years and above were significantly associated with development of DHF/DSS; the OR was 2.37 (95% CI= 1.14-4.94). However, no significant association was noted for variables such as gender and race (Table I).

Analysis of the symptoms and signs with which these patients presented showed no significant association for variables such as blood pressure, vomiting, symptoms of upper respiratory tract infection, abdominal pain, hepatomegaly, ascites and pleural effusion and development of DHS/DSS. The only significant symptom associated with DHF/DSS was diarrhoea; the odd ratio was 2.41 (95% CI = 1.04-5.57) (Table II).

Laboratory test analysis showed patients with secondary dengue infections were significantly associated with the development of DHF/DSS when compared to the group with primary infection (OR= 2.27; 95% CI= 1.08-4.78). So was the group with platelet count less than 35,000/mm3 (OR= 2.73; 95% CI= 1.26-5.89). On analysing the association of haematocrit with DHF/DSS, the results showed strong association of development of DHF/DSS with haematocrit values of 47% and above for male (OR= 13.22, 95% CI= 3.35-52.20), and 40% and above for female patients (OR= 3.96, 95% CI =1.52-10.33). It was also found that haematocrit fluctuation of more than 20% is highly significant in association with DHF/DSS, the odd ratio was 39.71 (95% CI= 13.90-113.48). The group with activated partial thromboplastin time (APTT) ratio of 1.25 or more was also found to have significant association with DHF/DSS (OR= 2.82, 95% CI=1.15-6.90), whereas international normalized ratio (INR) of prothrombin time (PT) was found to have no significant association with development of DHF/DSS (Table III).

Only 40 patients in our study population had liver function tests done during their admission. Though we were not able to prove significant association between the liver enzymes and bleeding tendency, the median aspartate aminotransferase (AST) was found to be higher in the group with DHF/DSS as compared to the group with Classical Dengue (207 IU/l and 119 IU/l respectively). The same went for ALT (114 IU/l and 75 IU/l respectively).

High haematocrit (OR=1.15, 95% CI=1.03-1.30) and APTT ratio of 1.25 and above (OR=2.75, 95% CI=1.06-7.15) were found to have independent significant association with DHF/DSS on multivariate analysis (Table IV).

Stratified analysis between age and types of infection revealed patients with age of 30 years and above with primary infection was significantly associated with DHF/DSS (OR =4.24, 95% CI = 1.40-12.84), on the other hand, age below 30 years with secondary infection was also significantly associated with DHF/DSS (OR =3.86, 95% CI = 1.29-11.58).

DISCUSSION

The identification of risk factors of DHF/DSS has its implications for clinical practice. It helps the physicians prioritize care during an outbreak of dengue infection. Besides, it offers a clue to the study of the pathogenesis of dengue infection.

Effect of age on severity of dengue infection studied by previous workers showed that infants and young children were at higher risk of developing DHF/DSS[13,14]. Guzman *et al* had demonstrated a bipolar pattern of increased mortality, whereby young infants and the elderly aged 50 years and older had higher case fatality and hospitalization rates[15]. Our study, being an adult study, showed older age group being 2.37 times more likely to develop DHF/DSS than the younger age group.

Secondary infection has been well recognized as the more aggressive form of dengue infection in the past[16,17], particularly with DEN 2 virus[18]. Our data relating severity of disease with secondary infection (OR 2.27; 95% CI = 1.08-4.78) are in agreement with previous work. Wichmann O *et al*[6] in their study showed that secondary dengue infection was significantly associated with the development of DHF in children, but not in adults. Similarly, our data identified a significant association between age and types of infection in

Table I: Demographic data in subjects with confirmed dengue, comparing those classified as having Classical Dengue and DHF/DSS

Demographic Characteristics	DHF/DSS (n=38)	Classical Dengue (n=145)	OR (CI 95%)
Age (years)*			
>30	21 (29.2%)	51 (70.8%)	2.37 (1.14-4.94)
<30	16 (15.4%)	92 (85.2%)	
Gender			
Female	25 (26.0%)	71 (74.0%)	0.50 (0.24-1.05)
Male	13 (14.9%)	74 (85.1%)	
Race			
Malay	31 (20.9%)	117 (79.1%)	1.06 (0.42-2.65)
Non-Malay	7 (20.0%)	28 (80.0%)	

* Age was not known in 3 patients

Table II: Clinical Features in subjects with confirmed dengue, comparing those classified as having Classical Dengue and DHF/DSS

Clinical Features	DHF/DSS (n=38)	Classical Dengue (n=145)	OR (CI 95%)
Blood pressure on admission*			
Normal	33 (21.2%)	123 (78.8%)	1.88 (0.53-6.68)
Low (<90/60 mmHg)	3 (12.5%)	21 (87.5%)	
Vomiting			
Yes	15 (22.4%)	52 (77.6%)	1.17 (0.56-2.43)
No	23 (19.8%)	93 (80.2%)	
Diarrhoea			
Yes	11 (34.4%)	21 (65.6%)	2.41 (1.04-5.57)
No	27 (17.9%)	124 (82.1%)	
URTI symptoms			
Yes	6 (21.4%)	22 (78.6%)	1.04 (0.39-2.80)
No	32 (20.6%)	123 (79.4%)	
Abdominal pain			
Yes	8 (23.5%)	26 (76.5%)	1.22 (0.50-2.97)
No	30 (20.1%)	119 (79.9%)	
Hepatomegaly			
Yes	12 (26.7%)	33 (73.3%)	1.57 (0.71-3.44)
No	26 (18.8%)	112 (81.2%)	
Ascites			
Yes	1 (50.0%)	1 (50.0%)	3.89 (0.24-63.70)
No	37 (20.4%)	144 (79.6%)	
Pleural effusion			
Yes	0 (0.0%)	3 (100.0%)	χ^2 p values
No	38 (21.1%)	142 (78.9%)	

*There was no initial blood pressure recordings in 3 patients.

Table III: Laboratory investigations in subjects with confirmed dengue, comparing those classified as having Classical Dengue and DHF/DSS

Laboratory Investigations	DHF/DSS (n=38)	Classical Dengue (n=145)	OR (CI 95%)
IgM and IgG antibodiesa			
Secondary Infection	23 (28.4%)	58 (71.6%)	2.27 (1.08-4.78)
Primary Infection	14 (14.9%)	80 (85.1%)	
Platelet count on admission			
<35, 000/mm^3	15 (34.9%)	28 (65.1%)	2.73 (1.26-5.89)
≥35, 000/mm^3	23 (16.4%)	117 (83.6%)	
Haematocrit on admission for maleb			
≥47%	7 (53.8%)	6 (46.2%)	13.22 (3.35-52.20)
<47%	6 (8.1%)	68 (91.9%)	
Haematocrit on admission for femalec			
≥40%	16 (42.1%)	22 (57.9%)	3.96 (1.52-10.33)
<40%	9 (15.5%)	49 (84.5%)	
Haematocrit fluctuation > 20%			
Yes	24 (80.0%)	6 (20.0%)	39.71 (13.90-113.48)
No	14 (9.2%)	139 (90.8%)	
Highest APTT ratiod			
≥1.25	17 (34.7%)	32 (65.3%)	2.82 (1.15-6.90)
<1.25	10 (15.9%)	53 (84.1%)	
Highest INR, mediand	0.955	0.910	t-value

a There were 8 undetermined cases whereby IgG was not performed. N=175.
b N=87
c N=96
d Only 112 patients had coagulation profile performed on admission, N=112.

Table IV: Multivariate analysis of univariately significant risk factors for DHF/DSS

Risk factors	Odds ratio (95% CI)	p value
Age	1.80 (0.67-4.83)	0.25
Secondary Infection	0.70 (0.25-1.94)	0.50
Haematocrit on admission	1.15 (1.03-1.30)	0.02
Platelet on admission	1.00 (0.99-1.01)	0.53
APTT ratio	2.75 (1.06-7.15)	0.04

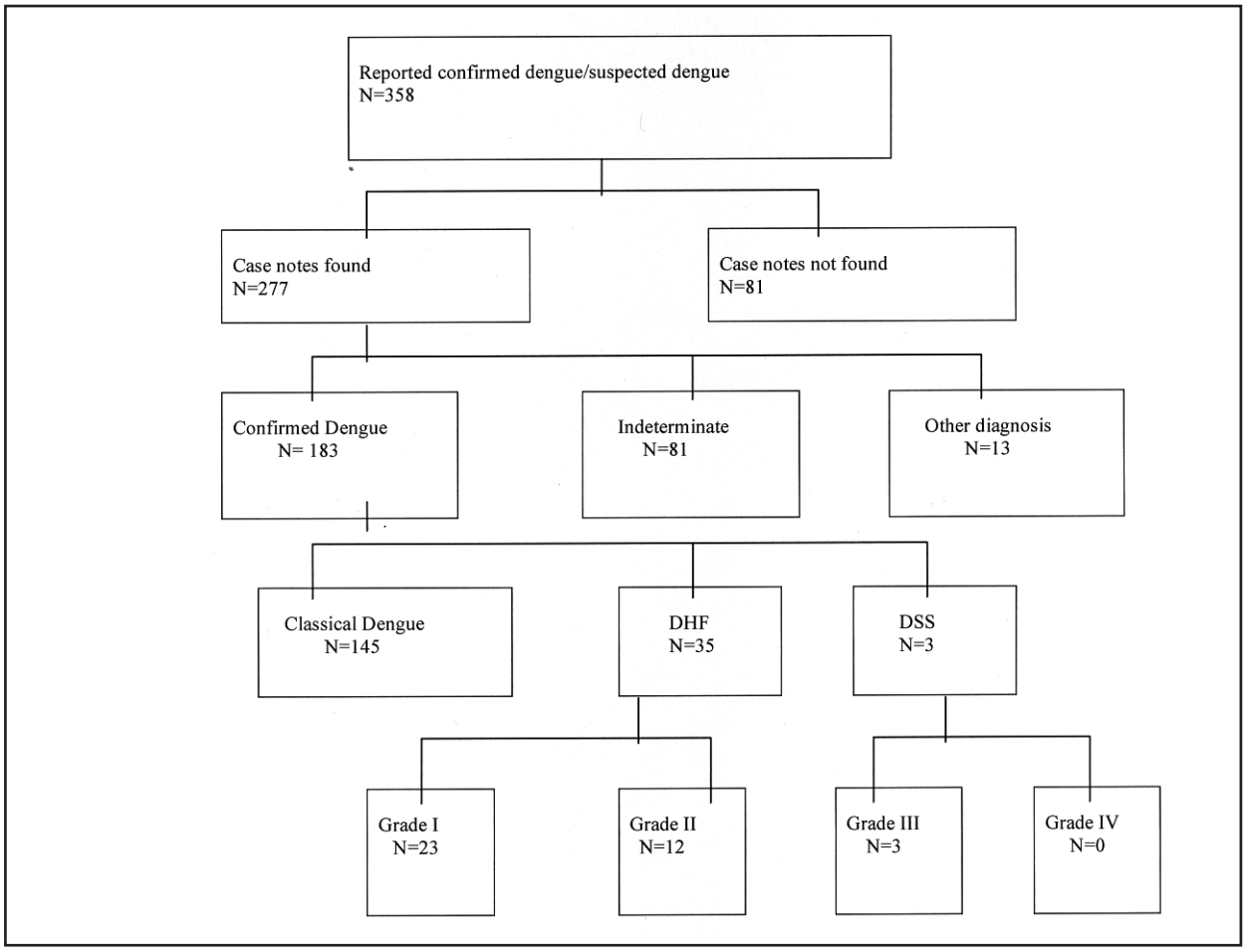

Fig. 1: Schematic diagram showing the final disease classification for all patients enrolled in the study

relation to development of DHF/DSS. Our study revealed secondary infection was actually more severe in patients with age below 30 years (OR=3.86, 95% CI=1.29-11.58). For patients age 30 years and above, it was primary infection that was more severe (OR= 4.24, 95% CI= 1.40-12.84).

Haematocrit has been used as an important parameter in monitoring patients with dengue infection. There were previous studies identifying cut-off values for the local population in defining DHF. Cao XT *et al* in 2004 found the median haematocrit value for DHF in Vietnamese children being 48%[19]. Gomber S *et al* in 2001 defined the haematocrit value diagnostic of DHF in Indian children at 36.3%, with sensitivity and specificity of 60% and 94%[20] respectively. In our study we concur with Malaysia's Clinical Practice Guidelines for Dengue Infections in Adults[21], in which haematocrit values of 47% for male and 40% for female were suggested for cut-off value to suspect plasma leakage. In our study, we had validated that these values are useful to determine risk of DHF/DSS. We also found that a fluctuation of 20% in the haematocrit values has highly significant association with DHF/DSS (OR= 39.71). However, clinical usefulness may be limited by the duration needed to observe the drop in haematocrit of more than 20%.

Dengue infection causes deranged coagulation profile in which the APTT is affected more that the PT[22,23]. Chua MN *et al* advocated APTT of >30 seconds to be used as an index in predicting bleeding in DHF[24]. Increased PTT and hypofibrinogenemia are a result of stimulation of intrinsic coagulation pathway probably from immunologic reaction. Although no significant decrease in coagulation factors were recorded[23], high level of von Willebrand factor was noted in DHF, especially during the early phase of the disease[25]. In our

study, we demonstrated significant association of prolonged APTT with the development of DHF/DSS (OR= 2.82, 95% CI=1.15-6.90), in agreement with other published studies.

The authors strongly felt that raised liver enzymes, especially aspartate aminotransferase (AST) may be a significant risk factor for DHF/DSS, as demonstrated by many studies in the past[23,26]. However, in our study, there were only 40 patients with confirmed dengue had liver function tests. The analysis of these limited data revealed the group with DHF/DSS had higher median ALT and AST. However these results were not statistically significant.

We were also not able to conclude that the risk factors for DHF/DSS studied could be extrapolated to predict mortality, as the number of mortality in the study period was too small (3 deaths out of 183 confirmed dengue). Additional studies with larger cohorts will be necessary to further investigate the associations between these risk factors and mortality.

CONCLUSION

Haematocrit, APTT ratio, secondary dengue infection in age below 30 years old and primary dengue infection in age 30 years old and above are significant risk factors in predicting occurrence of DHF/DSS. These factors could be utilized to formulate a predicting score to identify potential DHF/DSS in future outbreaks in order to prioritize care and reduce mortality.

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