A Review of Dengue Research in Malaysia

W K Cheah, MRCP*, K S Ng, MRCP, Abd-Rahman Marzilawati, MMed**, Lucy C S Lum, MRCP*****

*Department of Medicine, Hospital Taiping, 34000 Perak, **Department of Medicine, Hospital Kuala Lumpur, 50586 Kuala Lumpur, ***Department of Paediatrics, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur.

SUMMARY

Dengue infection is a major cause of morbidity and mortality in Malaysia. To date, much research on dengue infection conducted in Malaysia have been published. One hundred and sixty six articles related to dengue in Malaysia were found from a search through a database dedicated to indexing all original data relevant to medicine published between the years 2000-2013. Ninety articles with clinical relevance and future research implications were selected and reviewed. These papers showed evidence of an exponential increase in the disease epidemic and a varying pattern of prevalent dengue serotypes at different times. The early febrile phase of dengue infection consist of an undifferentiated fever. Clinical suspicion and ability to identify patients at risk of severe dengue infection is important. Treatment of dengue infection involves judicious use of volume expander and supportive care. Potential future research areas are discussed to narrow our current knowledge gaps on dengue infection.

KEY WORDS: *Dengue, Malaysia, serotypes, epidemiology, economic burden, diagnosis, prevention, vaccine*

SECTION 1: REVIEW OF LITERATURE

EPIDEMIOLOGY

Demographic

The incidence of dengue has increased dramatically around the world in recent decades. World Health Organization (WHO) estimated about 2.5 billion people (two-fifths of the world's population) were at risk for dengue. Today, the disease is endemic in more than 100 countries, including Africa, America, Eastern Mediterranean, South East Asia, and the Western Pacific. Among these regions, South East Asia and the Western Pacific are the most seriously affected[1]. Prior to 1970, there were only nine countries in the world that experienced dengue epidemics. By 1995, this increased four-fold[1]. In Malaysia, dengue cases have increased since the first major outbreak in 1973[1].

There were several published papers recording the number of dengue cases and dengue incidence rate since 1982[2-9]. From the unpublished data from dengue surveillance system, Vector Borne Disease Section, Ministry of Health (MOH) Malaysia, there is a trend of increasing cases of dengue from 16,368 cases in 2001 to 46, 171 cases in 2010. The sudden drop of cases in 2011 could be due to the methodology difference in case reporting and need further exploration (Fig I).

Dengue infections affected all age groups, gender and ethnicity. In a one-year retrospective study in Negeri Sembilan in 2010, involving 1,466 cases of dengue infection, the youngest affected was 8 months old and the oldest was 89 years old. The mean age was 32.2 ± 15.8 years old. In terms of ethnic groups, majority who were affected were Malays, followed by Chinese and Indians (Ratio of 4.1:1.5:1). More males were affected than females (Ratio 1.4:1.0)[10]. The pattern of male predominance was observed consistently over several years across six culturally and economically diverse countries in Asia[11]. In Malaysia, as in Singapore[12] the incidence of dengue among the paediatric population has been declining while the incidence in the adult population has been on the rise. In 2006, about 80% of reported dengue cases in Malaysia were in the > 15 years age group Ministry of Health (MOH). Dengue became one of the leading causes of hospital admission among adults.

Distribution of reported dengue cases were more concentrated in urban areas. As shown in a Negeri Sembilan study in 2010, the highest number of dengue cases (81.9%) was reported from Seremban, its largest city. Of these, 8.3% were dengue haemorrhagic fever[10]. Since most reported dengue cases were from urban areas, the seropositivity rate of dengue IgG in healthy volunteers would be expected to be higher in subjects from urban areas compared to those from the rural regions. A cross-sectional epidemiological study of dengue IgG seroprevalence in 1000 Malaysian adult population (35 to 74 years), showed a high prevalence of up to 91.6%[13]. Among these subjects, however, the seroprevalence rates between urban and rural areas were similar. Chen WS et al. reported a lower prevalence of seropositivity (76.5%) in a smaller study (39 females, 46 males, with mean age of 42.8 years, from Dec 2000 to Dec 2001)[14].

Dengue virus and serotypes

Dengue virus is a single stranded RNA virus. It belongs to the family of *Flaviviridae*, which comes from the genus of Flavivirus. It contains three structural proteins, namely capsid protein C, membrane protein M, and envelope protein E. Seven nonstructural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. At the time of writing of this article, five serotypes of dengue viruses have been identified. However, for the purpose of this review article, only four serotypes will be discussed.

All four dengue serotypes (DEN 1, 2, 3 & 4) could be isolated in Malaysia at any point of time. The simultaneous presence of all four serotypes indicates that Malaysia is "hyperendemic" for dengue. However different serotypes have predominated throughout the years. From 1992 to 1995, and in 2001-2002 DEN 3 predominated[15]. DEN 2 and then DEN 1 predominated

Corresponding Author: drcheah.moh@1govuc.gov.my

over the period of 1998-2000 and 2004-2006 respectively[4]. DEN 4 was once the predominant serotype in Malaysia from 1967-1969. After that period, DEN 4 occurred at low levels (less than 5%) for many years until 2001, when there was a slight increase[16]. The latest publication from the Institute of Medical Research (IMR) showed that DEN 3 was once again the predominant serotype for the years 2008 and 2009[17].

In a phylogenetic study of the DEN 2 strains that caused two major outbreaks in the country in 1990s, two different DEN 2 genotypes were identified: DEN 2 Asian 1 and DEN 2 Cosmopolitan. Eighty percent of the isolates were DEN 2 Cosmopolitan, which was further divided into Clade I and Clade II. The latter was responsible for two major outbreaks in the 1990s. These strains originated from the same ancestral lineage, suggesting that both came from the same DEN 2 gene pool[18] .

A surveillance data analysis of dengue serotypes in Negeri Sembilan over a 1-year period in 2010 showed the presence of all serotypes. DEN 3 the predominant serotype in January, co-existed with DEN 2 until May. Thereafter, DEN 1 was the predominant serotype[10] .

Other than circulation of the viruses from within the locality, one study showed that there were multiple entries of DEN 2 and DEN 4 into Sarawak. These isolates were closely linked to those circulating in different localities in South East Asia from 1997 to 2002, based on phylogenetic analysis. Nonetheless, there was little exportation out of Sarawak[19].

PATHOGENESIS

The pathogenesis underlying the wide spectrum of clinical presentations of dengue is not well understood. The risk of Dengue Hemorrhagic Fever (DHF) was higher in situations of hyperendemicity when two or more virus serotypes were circulating simultaneously. The presence of pre-existing dengue antibodies, either by prior infection or passive immunity via maternal antibodies, would in fact, enhance viral infectivity and multiplication leading to a higher viral load. In primary dengue infection, dengue virus attaches to the target cell via highly sulphated glycosaminoglycan heparin sulfate, from which it penetrates target cells via high affinity receptor. In contrast, in secondary dengue infection, dengue virus mediates its entry into target cells via Fcγ-receptors. This phenomenon is called antibody-dependent enhancement (ADE). The virus combines with specific antibody, creating a complex that is taken up by mononuclear cells, dendritic cells and B-lymphocytes via a FcR mediated endocytosis[20].

A post-mortem study by Jessie K *et al.* reported that viral antigens but not viral RNA were found in Kupffer and sinusoidal endothelial cells of the liver, alveolar macrophages, multinucleated cells, vascular endothelial cells, macrophages and vascular endothelium in the lung and kidney tubular cells. The absence of strongly positive immunohistochemistry signals in these cells indicated absence of replication activities. There was no evidence of the involvement of megakaryocytes in the bone marrow at the time of death. Therefore thrombocytopenia during the later acute phase of dengue is probably not related to failure of platelet production[21]. Another post-mortem study demonstrated that dengue RNA was not retrievable from the brain tissue, suggesting that dengue virus replication in the central nervous system is rare in the later stages of disease[22]. In another work, Fong MY *et al.* analysed four encephalitogenic DEN 3 isolated in 1996, and compared them with five non-encephalitogenic DEN 3 viruses. Their envelope protein E showed high degree of similarity, suggesting that the neurovirulence of encephalitogenic dengue virus was not attributed to their envelope protein[23].

To further understand the pathophysiology of dengue infection, the investigation of various immune parameters, cytokines and antibody was reviewed by Shamala D[20]. Seventeen different peptides, C, E, NS2B, NS3, NS4A, NS4B and NS5 regions were found to evoke significant response in T cells of patients with dengue infections. Indeed, at the defervescence stage, viral load falls abruptly. It could be the response of the T cells to various stimuli that lead to tissue damage and severe immune response, followed by vascular leakage, bleeding and shock[24]. A study on the effect of active cytokines in the serum of DHF patients on human umbilical vein endothelial cells demonstrated changes in the vascular endothelium. The authors suggested that the production of cytokines during dengue significantly enhanced vascular permeability. However, this vascular permeability effect was transient[25]. At the febrile phase, IP-10 and MIP-1b were significant in dengue patients, with and without warning signs. At this stage, only MIP-1b was found to be significant in patients with warning signs. The IP-10 together with MIP-1b, G-CSF and MCP-1 were significant in patients during defervescence. Significant correlations between different cytokines group and the level of blood leukocytes, platelets and liver enzymes could be seen[26].

Genetic markers might be implicated in predicting susceptibility and/or protection to severe clinical manifestation of dengue infection. HLA-B*53 probably conferred susceptibility to DHF, while the HLA-A*03 and HLA-B*18 might confer protection from progression to severe disease. Interestingly, in the Malay subgroup, HLA-B*13 and B*18 were probably associated with disease susceptibility and protection, respectively[27].

PRESENTATIONS

The clinical presentation in the early febrile phase of illness is that of an undifferentiated fever. The prevalence of individual symptoms varied from one report to another. The most common symptoms were fever (100.0%), followed by headache (27% - 100%), myalgia and athralgia (39%- 99%), and nausea and vomiting (38%- 54%). Less common symptoms were rash (18-24%), petechiae and bleeding tendencies (7.0%-62%), and neurological deficits (1.2%). Abdominal pain and tenderness, gastrointestinal bleed, jaundice, hepatomegaly and ascites were predictors of the need for intensive care as reported by Ooi ET *et al.*[28]. Additionally, hepatomegaly and liver dysfunction were more common in DHF than Dengue Fever (DF)[29].

The tourniquet test, recommended by the World Health Organization (WHO) had a sensitivity of 82.8% and a specificity of 23.5%[30]. The positive predictive value (PPV) was 70.7% and negative predictive value (NPV) was 28.6%[30,31]. The presence of atypical reactive lymphocytes was seen in 85% (23/27) of patients with dengue fever[32]. However, the full blood picture was not a routinely requested investigation in the management of dengue, neither was laboratory confirmation of dengue.

In an outpatient setting in a dengue endemic area, thrombocytopenia in the context of an undifferentiated acute febrile illness (AFI) had a sensitivity of 88% and specificity of 71% to predict acute dengue infection. Thrombocytopenia was more useful to exclude than to diagnose dengue infection[33-35]. On the other hand, there was considerable overlap in clinical features of those with dengue infections with those with other AFI[36].

A cross-sectional retrospective study of 121 DHF children admitted to Hospital Kuala Lumpur from January 1999 to May 2001 reported fever in all patients. Vomiting and mild bleeding were observed in almost half of the children. Severe gastrointestinal bleeding was observed only in those with profound shock. Evidence of plasma leakage (pleural effusion and/or ascites) was present in 57% of the cases. Lowest mean platelet count was 41,785 /uL on the 6th day of illness. Hyponatraemia was a significant electrolyte imbalance seen in 65.9% of the cases[37].

In a single-centre outpatient-based prospective observational cohort study enrolling 214 patients >16 years with < 72 hours of undifferentiated fever, 65% eventually had a laboratory confirmed diagnosis of dengue, the rest were classified as other febrile illnesses (OFI)[38]. Of the 140 dengue patients, 11.4% developed DHF, no patients developed Dengue Shock Syndrome (DSS) and 37.1% required hospitalisation. In addition to a recent history of dengue within the family or neighborhood, the three early clinical predictors of dengue at < 72 hours of fever were: nausea and/or vomiting, postural dizziness and lower total white cell count compared to patients with OFI. Symptoms frequently reported by dengue patients such as headache, myalgia, arthralgia and retro-orbital pain were also observed in patients with OFI, with no significant differences between the two groups.

Chikungunya infection is spread by the same vector *Aedes aegypti* mosquito. Both dengue and chikungunya cause similar clinical features such as fever, myalgia, headache, arthralgia, and rash. Compared to patients with chikungunya infection, dengue patients were generally younger. A retrospective study of 60 patients with chikungunya and 120 patients with dengue from April 2008 to July 2009 in University Malaya Medical Centre, showed that the former was independently associated with arthralgia and rash, while latter was associated with myalgia, raised aspartate transaminase and leucopaenia. Arthralgia was seen in about 96% of chikungunya and 30% of dengue. Hence arthralgia is a strong predictor for chikungunya infection; self-reported arthralgia (22.5%) was reported for up to 1 year of follow up[39].

Tan PC *et al.* in their study involving 411 patients presenting with miscarriage, found 11 of the subjects to have dengue IgM or NS-1 Ag positive. The sample size was, however, too small to support the correlation between recent dengue infection and early pregnancy outcome[40]. Maternal and neonatal outcomes in dengue IgM seropositive women at delivery were not affected by subclinical recent dengue infection. Rates of preterm birth, mode of delivery, postpartum haemorrhage, low birth weight, and neonatal outcomes were not increased. The prevalence of the maternal seropositive rate was 2.5% (63/2531) with one case of vertical transmission rate, 1.6% (1/64)[41].

Several studies have attempted to describe clinical predictors of severe dengue[42,43].All of the ten dengue deaths reported by UMMC from June 2006 till October 2007, had secondary dengue infection (dengue IgG positive and dengue NS-1 positive, or dengue IgG positive in less than two weeks of infection). Clinical and laboratory warning signs of severe dengue included vomiting (90%), diarrhoea (60%), bleeding (80%), and evidence of vascular permeability (60%). Five patients had severe bleeding (gastrointestinal, lungs, brain or per vaginal) (50%), elevated liver enzymes (ALT>1000 IU/L) (50%) and hypoalbuminaemia (70%)[42].

Haemophagocytic syndrome has been reported in patients whose duration of fever, cytopenia and multi-organ dysfunction were prolonged beyond the plasma leakage phase of illness. This under-recognised phenomenon is most probably due to a "cytokine storm", caused by activated macrophages that secrete large amount of inflammatory cytokines. Diagnosis should be confirmed by bone marrow examination. Treatment should be supportive; however in severe cases, may include high dose immunosuppressive therapy such as IV methylprednisolone[44] and intravenous immunoglobulin. Other rare presentation of dengue infections include prolonged thrombocytopenia[45], myositis[46] and maculopathy[47,48].

Rare complications of dengue fever have been described. Most of these are believed to be immune-mediated. Three main mechanisms have been postulated to explain atypical neurological manifestations: direct neurotropic invasion, systemic complication and post infectious immune mediation[49]. Post-dengue associated Parkinsonism was reported by Azmin *et al.*[50] in which an 18-year-old man with NS-1 positive dengue developed Parkinson-like features, multiple cranial neuropathies, cerebellar ataxia and brachial plexopathy at day 9 of the illness. He was treated with IV methylprednisolone 500mg daily for 3 days. At one-month review, his symptoms of Parkinsonism and cerebellar ataxia had resolved but weakness in the right deltoid and infraspinatus muscles remained, together with marked muscle atrophy. Electromyogram (EMG) revealed chronic denervation changes involving the right deltoid and right trapezius muscle, which points to right brachial plexopathy. This is the second reported case of dengue-fever associated brachial plexopathy. Cerebellar ataxia following dengue fever has also been reported[50]. In the absence of dengue viral antigen in the cerebrospinal fluid coupled with CSF pleocytosis, the mechanism points towards immune mediation[50]. Other complications include dengue encephalitis[51] and atrial fibrillation[52].

DIFFERENTIAL DIAGNOSIS

The nonspecific clinical features of dengue may mimic many acute febrile illnesses such as acute flu-like syndrome, acute rash syndrome, acute diarrhoeal syndrome and acute neurological syndrome. On the other hand, during the critical phase when fever subsides, dengue may mimic acute abdominal conditions, acute respiratory conditions, septicaemic shock and any condition with leucopenia, thrombocytopenia and bleeding.

A multicentre prospective study identifying the causes of acute febrile illness in the paediatric group (2-14 years old) in South East Asia found the most common causes in Malaysia to be dengue fever, chikungunya and influenza A. Other tested illnesses included S. Typhi, rickettsia and hepatitis A[53]. Hamidon BB *et al.* reported a case of Seoul hantavirus infection that presented with haemorrhagic fever mimicking dengue infection. Seoul hantavirus is carried by domestic rats. This condition should be considered in dengue-sero-negative patients with clinical haemorrhagic features mimicking dengue[54].

A single positive dengue IgM should not be considered confirmatory as illustrated by a case[55] of a 47-year-old man from Pakistan, who was reported to have a mixed infection of leptospirosis and *vivax* malaria, despite a positive dengue IgM which could be explained by a recent dengue infection in the past 3 months. On the other hand, confirmed co-infection of dengue with other infection such as chikungunya has also been described[56].

DIAGNOSIS

The WHO case classification (1997) for dengue was used to differentiate DF from DHF/DSS. The DHF cases must fulfil all four of the following criteria:

1) Fever or history of acute fever lasting 2–7 days.
2) Haemorrhagic tendencies evidenced by at least one of the following:
   a) A positive tourniquet test. The test may be negative or mildly positive during the phase of profound shock. It usually becomes positive, sometimes strongly positive, if the test is conducted after recovery from shock (this limits its clinical usefulness);
   b) Petechiae, purpura, ecchymoses;
   c) Bleeding from mucosa, gastrointestinal tract, injection sites or other location haematemesis or melena.
3) Thrombocytopaenia (100,000 platelets/µl or less)
4) Haemoconcentration (20% or more rise in the haematocrit value relative to baseline average for the same age, sex and population) or evidence of plasma leakage (i.e. pleural effusion, ascites and/or hypoproteinaemia).

Ng CF *et al.* studied clinicians' ability to classify dengue using the WHO 1997 classification in a university hospital. They observed that DHF/DSS was under-recognised by clinicians. Out of the 520 adult and 191 paediatric hospital records that were reviewed, thrombocytopenia and evidence of plasma leakage were present in 8% of adult and 19% of paediatric patients. Of these patients who fulfilled the criteria for DHF, 93% and 49% respectively, were discharged with a diagnosis of DF[57].

A retrospective study by Tee *et al.* in Hospital Tengku Ampuan Afzan Kuantan between October 2004 and March 2005 involving 183 cases of confirmed dengue was conducted to evaluate the clinical and laboratory findings that correlated with the development of DHF or DSS. Seventy nine percent (145 cases) were classical dengue, 19% (35 cases) were DHF and 2% (3 cases) were DSS[42]. Table I is a summary of risk factors identified in this study.

Risk factors for haemorrhage in 114 children with DSS were hypotension, mottling, encephalopathy, organ failure, prolonged duration of shock, abnormal glycaemia, normal-low haematocrit at the diagnosis of shock, and abnormal coagulation ($P < 0.05$). However, the independent risk factors for haemorrhage in this cohort of DSS children were duration of shock (OR, 2.11; 95% CI, 1.13 to 3.92; $P = 0.019$) and normal-low haematocrit at the time of shock (OR, 0.72; 95% CI, 0.55 to 0.95; $P = 0.020$). On the other hand, platelet counts were not predictive of bleeding[58].

Bandyopadhyay S *et al.* in their review of 37 articles, reported that most clinicians reported difficulties in meeting all four WHO criteria for DHF/DSS and used a modified classification[59].

The positive tourniquet test representing the minimum requirement of a haemorrhagic manifestation did not distinguish between DHF and DF. In cases of DHF, thrombocytopenia was observed in 8.6–96%, plasma leakage in 6–95% and haemorrhagic manifestations in 22–93%. The low sensitivity of classifying DHF could be due to failure to repeat the tests or physical examinations at the appropriate time, early intravenous fluid therapy, and lack of adequate resources in an epidemic situation and perhaps a considerable overlap of clinical manifestations in the different dengue entities.

Some of the limitations of WHO classifications are as follows[59]:

1) Dengue with shock without fulfilling all four criteria of DHF
2) Severe organ impairment with or without shock are not captured
3) Haemoconcentration is hard to define in patients without prior baseline hematocrit.
4) Not useful in terms of clinical management

In view of the above limitations, a revised WHO classification in 2009 classifies dengue illness into dengue fever with/without warning signs and severe dengue. Severe dengue is characterised by severe plasma leakage, severe haemorrhage and/or severe organ impairment.

Faisal T *et al.* suggested an alternative risk criteria derived using the self-organised map (SOM) method. A patient fulfilling any two of the risk criteria is considered a high risk dengue patient. The criteria included:

a) Platelet count less than or equal 40,000 cells per mm^3,
b) Haematocrit concentration greater than or equal 25% rise, and
c) Aspartate aminotransferase (AST) rise of five times the normal upper limit for AST/alanine aminotranstansferase (ALT) rise of five times the normal upper limit for ALT[60]. The usefulness of SOM in clinical management has yet to be identified.

Diagnostic tests

The laboratory confirmation of dengue is a challenging issue. Fig.3 could be a guide on the most appropriate tests to diagnose the infection based on the day of illness and whether the infection is a primary or secondary. Research related to diagnostic tests, done locally or internationally are not reviewed and will not be further discussed here.

Other non-conventional test

Bioimpedance analysis (BIA) of water content in different body compartments of dengue patients and healthy subjects[61,62] was only able to explain approximately 42% of the variation in serum haemoglobin status, thus limiting its usage as a surrogate monitoring system for haemoglobin and haematocrit levels[62].

Another study used the multilayer feed-forward neural networks (MFNN) to predict dengue patients' defervescence phase which is solely based on clinical symptoms and signs. This system has a 90% prediction accuracy[63]. Combining BIA and artificial neural network (ANN), Ibrahim F *et al.* was able to show that ANN provided a system that was able to classify and diagnose patients with risk of severe dengue infection with an overall accuracy of up to 96.27%. The disadvantage of these systems is that they may appear too technical for clinicians[64].

Thayan R *et al.* showed that both alpha1-antitrypsin and NS1 proteins were overexpressed by two-fold in DHF patients compared with DF patients[65]. By analysing levels of protein expression in peripheral mononuclear cells, Thayan R et al. showed that alpha tubulin and thioredoxin peroxidase were over-expressed by 4.9 times in DHF patients and 3.3 times in DF patients, while aldolase was up-regulated by 2.2 times in DF patients compared to DHF patients[66]. The role of these biomarkers as indicators for DHF in dengue infected patients is worth exploring.

In short, management of dengue involves management of fever and adequate oral fluid intake during the febrile phase, usually the first 3 to 4 days of illness. For more details on

outpatient management, please refer to the following youtube videos:

http://www.youtube.com/watch?v=p6XPWAco958 – Patient-doctor encounter
http://www.youtube.com/watch?v=tPLLJ2Dka_k – dengue on the rise

After the first 3 to 4 days of illness, monitoring the patient for warning signs of plasma leakage, haemorrhage and shock becomes the main focus of management. Management of dengue shock involves judicious volume replacement with isotonic crystalloids and colloids and supportive care. In a randomised, double-blind comparison study done by Dung N.M. *et al.*[67], Dextran 70 (a colloid) gave the most rapid normalisation of haematocrit, with restoration of cardiac index, without adverse effect. There has been, however, no study on intravenous fluid management in adult patients with dengue shock.

The greatest challenge of intravenous therapy in dengue shock is to give "just enough" to maintain a "good enough" circulation without excessive fluid overload which leads to difficulties in breathing. Preventive transfusions of packed cells and fresh frozen plasma in paediatric patients with dengue shock syndrome with abnormal laboratory coagulation profile without bleeding are not necessary[68].

The study of Carica papaya leaves juice in patients with dengue infection shows a clinically modest but statistically significant rise of platelet count after 40 hours of ingestion of the juice[69]. This study was, however, not designed to address the two critical issues in dengue case management: its efficacy when used in the early febrile phase and whether drinking the papaya leaf juice could prevent the more critical complication of plasma leakage.

A case report of fulminant liver failure in an eight-month-old infant highlighted the potential of excessive dosing of paracetamol to cause this complication[70]. This should serve as a cautionary in adults too.

SPECIAL COMPLICATIONS

Acute liver failure is known to complicate severe dengue. However, the pathogenesis is not well understood. Acute liver failure could be a direct effect of severe dengue infection or a result of a secondary bacterial/fungal infection complicating the dengue infection. A retrospective case series of eight patients with DHF and acute liver failure (ALF) was reported by the national hepatology referral centre (Hospital Selayang, Department of Hepatology). Six of the eight cases had secondary bacterial / fungal infection in the blood and all had systemic inflammatory response syndrome (SIRS). All patients received broad spectrum antibiotics and some patients received fluconazole[71]. Although N-acetycysteine infusion was given as a routine in this report, it should be noted that this practice is not evidence-based.

POTENTIAL ANTI-VIRAL AGENTS

The development of antiviral therapy against dengue infection addresses targets that affect viral to host cell attachment, viral entrance or replication. Local research were restricted to laboratory tests on identifying potential candidates for antiviral development, such as NS5 MTase[72] (methyltransferase (MTase) enzyme which is responsible for assisting viral attachment to host cell via methylation of the viral RNA cap structure) and NS2B/NS3 protease[73] (enzyme responsible for viral life cycle via polyprotein processing).

HEALTHCARE SETTING AND ROLE

The role of primary care physician is important not only for early case detection and management, but also to promote preventive measures in the patients contact environment as well as notification for preventive measures to be taken by the state district health office. Ang KT *et al.* reported that 83.9% of hospitalised dengue patients have sought medical consultation at primary care facilities before admission to hospital and 68.7% had been seen on two or more occasions. The mean duration between first contact with primary care and hospitalisation was 1.4 days. Up to 98% of the patients reported that they had not been not advised on preventive measures even though 51.9% had been informed that they could be having dengue[74] .

A short stay in the emergency department could be an alternative to limit the burden of dengue inpatients while serving as a safety net for untimely discharge and unnecessary admissions. In a retrospective study done in University Kebangsaan Malaysia Medical Centre (UKMMC) from January to March 2010, patients with suspected dengue, who stayed in casualty, had a mean total length of stay of 32.2 hours. All patients were discharged well[75].

PUBLIC HEALTH MANAGEMENT AND CONTROL OF INFECTION

Research related to public health management and control of dengue infection, done locally or internationally were not reviewed and will not be further discussed here.

VACCINATION

The trend of dengue outbreak is likely to continue in Malaysia unless there is a breakthrough in the dengue vaccine development[76]. Vaccine introduction is a complex process[77]. The Dengue V2V initiative was established in 2009 to act as a global scientific expert's forum to lay the groundwork for rapid dengue vaccine introduction. The first Asia-Pacific meeting was held in Singapore at the end of 2010. The experts recommended few key important points which included documenting the actual human and economic costs of dengue, ensuring reliable surveillance of disease, identifying countries or regions for initial vaccine implementation based on data available, developing local logistical plans for dengue vaccine introduction, implementing holistic educational programmes (for health care workers, decision makers and the public), identifying sustainable source of funding and for each countries to take ownership of the disease and redefine the global view on dengue[78].

An effective vaccination program with effective dengue vaccine will be most welcomed. This is based on the fact that:

1) Dengue poses significant disease burden,
2) Current control measures have limited efficacy,
3) Disease treatment is limited to supportive care but the outcome is good if timely care is given.

The information regarding dengue vaccine in this article is limited as we only reviewed published data about the vaccine in Malaysia between 2000 and 2013. A Phase III Randomised Controlled Trial (RCT) on safety and immunogenicity of a tetravalent dengue vaccine in children showed that there were satisfactory safety profile and a balanced humoral immune response against all four DEN serotypes via three dose regimen. Most adverse events were of mild intensity and transient. There was no death. At third dose, seropositivity against all four DEN serotypes increased between 6.1-7.96 fold from baseline across all serotypes[79].

A study that was conducted in two cycles of outbreaks involving dengue virus DEN 2 of the same genotype found that sera of patients from the first outbreaks had poorer neutralisation activity against virus of the second outbreak. There were mild amino acid changes on the viral envelope protein between the same genotypic viruses from two separate outbreaks[80]. This, in part, could explain the eight years cyclical outbreaks of homogenotypic dengue virus in Malaysia. Additionally, effective vaccine should consider the potential subtle antigenic changes that can occur within a homogenotypic dengue virus. Furthermore, from our experience with pneumococcal vaccine introduction, there is the potential for serotype replacemenent with dengue vaccine.

Teoh BT *et al.* and Cardosa MJ *et al.* report on recent local isolation of ancestral sylvatic DEN 1 and DEN 2 from two separate cases of infected humans[81,82]. However, unless there is evidence that there are flare up of slyvatic strains, immunity against the existing four serotypes via vaccination may be good enough.

ANTIBODY DEPENDENT ENHANCEMENT (ADE THEORY) AND DENGUE

In the context of vaccination, two questions on the ADE theory should be addressed:

(i) Would an individual who has yet to complete the full vaccination regime develop a more severe disease?
(ii) Would an individual with waning antibody levels to sub-neutralising levels sometime after vaccination develop more severe disease?

To date, no evidence of ADE phenomenon has been observed. However, continuous monitoring well beyond vaccine introduction, for at least 5 years is necessary. This is to ensure that the vaccinated population will not succumb to increased risk of severe dengue.

MORTALITY

Case fatality rates for dengue cases is in a reducing trend from 0.31 in 2001 to 0.16 in 2012 (Fig. 2). A report of a tertiary referral hospital revealed a 4.1% mortality rate among DHF paediatric patients[83]. Since 2008, all dengue deaths were reviewed at hospital and state health departments and finally at the national level. To date, however, there has been no published literature that report on factors that contributed to the mortality.

QUALITY OF LIFE (QOL)

Lum LCS *et al.* in a prospective study showed that all the 207 participants experienced a drastic decrease in their QoL from the onset of symptoms. The QoL was the lowest (40% of healthy status) between the third and seventh day of illness. The duration of impaired QoL was longer than the duration of fever: nine days for ambulatory patients and 13 days for hospitalised patients[84].

ECONOMIC BURDEN

A large study involving eight countries in America and Asia looked at the economic burden of this disease. The estimated cost of dengue in Malaysia was a mean of USD 317 ± 105 for an ambulatory case and USD 947 ± 389 for a hospitalised case. The average illness lasted 8.6 days for ambulatory patients and 12.6 days for hospitalised patients. Among hospitalised patients, students lost 2.2 days of school, while those working lost on average 6.7 work days per dengue episode[85]. In another study, it was estimated that the economic burden of dengue illness was USD 56 million per year, which is approximately USD 2.03 per capita. The annual expenditure was higher ranging from USD 88 million to USD 215 million if additional cost of managing dengue such as vector control and research and development were included[86].

Economic studies may, however, underestimate the burden of cost if adjustment for under-reporting is not made. Undurraga Y *et al.* stressed the use of expansion factors (EF) to estimate a more accurate burden of dengue in Southeast Asia (SEA). They estimated EF of 7.6 for South East Asia in general and 3.8 for Malaysia[87,88]. None of these studies included costs associated with dengue prevention and control, disease surveillance and long term sequelae of dengue. If these were included, the economic burden will be even greater.

HEALTH ECONOMICS OF DENGUE VACCINE

In view of the high economic burden of a dengue epidemic[85], an effective dengue vaccine might be cost-effective. However, more studies will be required to study the particular age-group to be targeted for vaccination.

SECTION 2: RELEVANCE OF FINDINGS FOR CLINICAL PRACTICE

Malaysia has all four dengue virus (DENV) serotypes that infect and circulate among humans.

There may be some genetic predisposition to or protection from severe dengue in certain patients. The findings of inflammatory markers both during the febrile phase and defervescence phase help us to understand the pathogenesis of dengue infection a little bit better. At present, the utility of genetic and biomarkers are still restricted to research purposes.

Clinicians should be aware of the non-specific nature and the wide spectrum of clinical presentation of dengue, and to consider it a possible or probable diagnosis in every case of undifferentiated fever. This has important implications on clinical management and anticipatory follow-up for severe disease and the clinical outcome. Tourniquet test has been shown to be neither sensitive nor specific. The revised WHO classification (2009) is more user-friendly in guiding management of a disease that not only has a diverse but evolving dynamic presentation. In this revised version, clinicians should look out for warning signs and risk factors and conditions known to be associated with severe disease. These include pregnancy, infancy, old age, diabetes mellitus, hypertension, chronic renal failure, chronic heart disease and chronic haemolytic conditions. The warning signs of severe vomiting, severe abdominal pain and lethargy may precede hemodynamic changes of significant plasma leakage.

The laboratory confirmation of dengue is a challenging issue. As Fig. 3 shows the most appropriate tests to confirm the infection which depends on the day of illness and whether the infection is primary or secondary. Dengue IgM is usually present between days 5 and 7 of illness. Therefore, a negative IgM earlier than day 7 does not exclude dengue infection while a positive test may not indicate a dengue infection. A negative IgM for dengue should be repeated during recovery phase. In primary and secondary dengue infection, dengue IgG can be detected in most patients after day 7of illness. Therefore, IgG is recommended after day 7 of illness if IgM is not detected and IgG done earlier was also negative. Dengue PCR is a useful diagnostic tool in early dengue infection, but the use is limited due to the cost and availability. NS 1 antigen is now increasingly used in the early phase of dengue infection.

Management of dengue infection currently focuses on fluid management but the treating physicians should be aware of the dangerous effects of fluid overload.

Table I: Risk factors associated with the development of DHF or DSS

Risk factors with DHF / DSS	OR (CI)
Patients aged > 30 years	2.37 (95% CI= 1.14-4.94)
Patients aged > 30 years with primary infection	4.24 (95% CI = 1.40-12.84)
Patients aged < 30 years with secondary infection	3.86 (95% CI = 1.29-11.58)
Diarrhoea	2.41 (95% CI = 1.04-5.57)
Patients with secondary dengue infections	2.27(95% CI= 1.08-4.78)
Platelet count < 35,000/mm3	2.73 (95% CI= 1.26-5.89)
Independent risk factors with DHF / DSS	
Haematocrit values of > 47% for male	13.22 (95% CI= 3.35-52.20)
Haematocrit values of > 40% for female	3.96 (95% CI =1.52-10.33)
Haematocrit fluctuation of more than 20%	39.71 (95% CI=13.90-113.48)
Activated partial thromboplastin time (APTT) ratio of > 1.25	2.82 (95% CI=1.15-6.90)

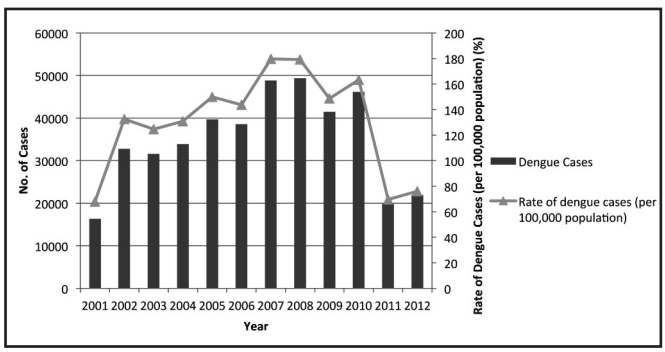

Fig. 1: Number of dengue cases and dengue incidence in Malaysia, 2001-2012.

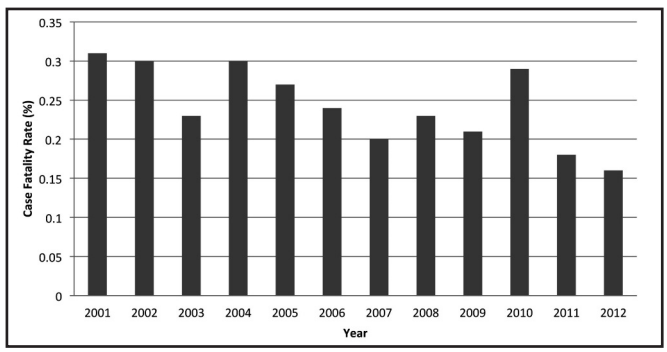

Fig. 2: Dengue Case Fatality Rate (CFR) in Malaysia, 2001-2012.

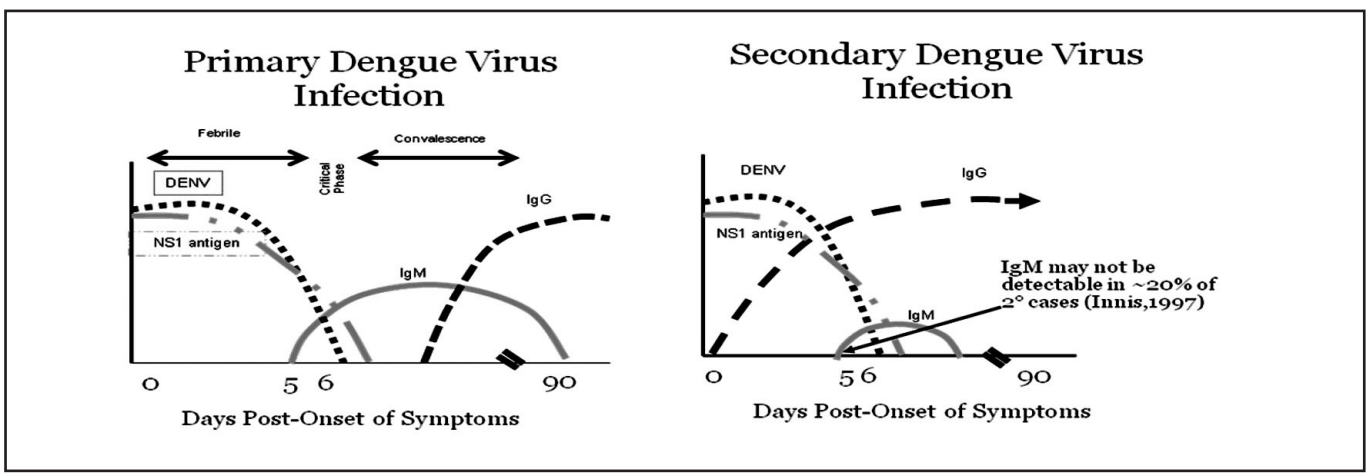

Fig. 3: Relationship between the phases during primary and secondary dengue infections with levels of dengue virus, NS1 antigen, IgM and IgG.

Unusual presentations should provoke considerations of secondary bacterial infections, co-infections with other pathogens, side-effects of drugs such as paracetamol or non-steroidal analgesics and the immune phenomena of haemophagocytosis and post viral infections. Unlike other viruses within the genus Flavivirus from the family Flaviviridae, dengue neurological complications whether due to encephalitis or encephalopathy appear to have good clinical outcome. However, on the rare occasion where dengue virus could be isolated from the brain tissue, the evidence does not support a direct virus invasion of the brain.

A therapeutic trial of the anti-viral effect of lovastatin in dengue infection is currently ongoing[89] and due to be completed in 2015. The phase 3 tetravalent dengue vaccine trial with a follow up period of 25 months[90] in children has an overall protection of 56.5%. Its protection in adults who bear the major burden of illness is still unknown. Other unanswered questions include the duration of protection and the effect of a new dengue serotype from the sylvatic cycle. The implementation of a vaccination programme requires thorough planning of logistics not least of which is a sustainable source of funding[79]. Unlike other arboviruses, dengue virus is maintained in the human cycle without replenishment from a zoonotic reservoir. Thus, wide spread use of dengue vaccine has the potential to eradicate human dengue infections. However, virus eradication by vaccination may provide an 'empty niche' which may eventually be filled by the adaptation of a related virus.

Both vector control and environmental planning may be the most effective methods to deal with the increasing number of dengue infection at this period of time. However the level of awareness of the environmental factors in dengue transmission among the public and their cooperation is still low.

Several local cohort studies showed that the level of awareness among the public with regards to dengue is still very low.

SECTION 3: FUTURE RESEARCH DIRECTION

The best way forward that will provide a more holistic understanding of the disease, clinical manifestations and pathogenesis is collaborative work among clinicians, epidemiologist, entomologist, primatologist and virologist.

The impact and cost-effectiveness of NS1 Ag in the evaluation of acute fever and the optimum outpatient management of dengue infection and admission criteria should be given priority by the primary care and emergency departments.

The effectiveness of and compliance to the current national guideline on acute management of dengue fever are topics of clinical importance. This includes studies looking at the appropriateness of fluid volume, type of fluids in different stages of dengue infection and the admission criteria. Effectiveness of oral fluid therapy in dengue patients should be evaluated. The effect of paracetemol on the liver function and the efficacy of N-acetycysteine in severe liver impairment due to dengue should be assessed. The potential effect of statin on dengue should be explored from a different perspective as we eagerly wait for the result form the Vietnam study[88].

The barriers to implementation of national dengue guidelines should be explored, particularly in view of the large percentage of deaths due to fluid overload. A comprehensive report of dengue deaths may alert clinicians of potential pitfalls in case management. By evaluating various training methods in dengue case management, effective teaching tools may be identified. A more sensitive method of staging the severity of dengue infection should be developed and validated to aid decision-making on outpatient or inpatient care.

ACKNOWLEDGEMENT

We would like to thank the Director-General of Health, Malaysia for his permission to publish this paper. We wish to thank the Clinical Research Centre team for its contribution and support.

REFERENCES

1. Wallace Hazel G, *et al.* Dengue haemorthagic fever in Malaysia 1973 epidemic. South East Asian J. Trop. Med. Pub. Health 1980; 11: 1-12.
2. Fang R, Lo E, Lim TW. The 1982 dengue epidemic in Malaysia: epidemiological, serological and virological aspects. Southeast Asian J Trop Med Public Health 1984; 15: 51-8.
3. Malaysia MoH. Clinical Practice Guidelines on Management of Dengue Infection in Adults. (revised 2nd ed)2010.
4. Malaysia MoH. Statement on dengue by director general of health. 2009.
5. Malaysia MoH. Annual report 1974,1980, 1984, 1998, 1999, 2000.
6. Mia MS, Begum RA, Er AC, *et al.* Trends of dengue infections in Malaysia, 2000-2010. Asian Pac J Trop Med 2013; 6(6): 462-6.
7. Arima Y, Matsui T. Epidemiologic update of dengue in the Western Pacific Region, 2010. Western Pac Surveill Response J 2011; 2(2): 4-8.
8. Arima Y, Edelstein Z, Han HK, *et al.* Epidemiologic update on the dengue situation in the Western Pacific Region, 2011. Western Pac Surveill Response J 2013; 4(2): 47-54.
9. Chew MH, Rahman MM, Sharifah Azura S. Dengue in Malaysia: An epidemiological perspective stud . Pak J Med Sci 2012; 28(4): 643-7
10. Ahmad Nizal MG, Rozita H, Mazrura S, *et al.* Dengue infections and circulating serotypes in Negeri Sembilan, Malaysia. Malaysian Journal of Public Health Medicine 2012; 12(1): 21-30.
11. Anker M, Arima Y. Male-female differences in the number of reported incident dengue fever cases in six Asian countries. Western Pac Surveill Response J 2011; 2(2): 17-23.
12. Ooi EE, Gubler DJ. Dengue in Southeast Asia: epidemiological characteristics and strategic challenges in disease prevention. Cad. Saúde Pública, Rio de Janeiro 2008; 25 Sup 1: S115-S124.
13. Nor Azila, MA Sharifah Azura S, Neoh HM, *et al.* Dengue epidemic in Malaysia: Not a predominantly urban disease anymore. BMC Res Notes 2011; 4(216): 0500-4-216.
14. Chen WS, Wong CH, Cillekens L. Dengue antibodies in a suburban community in Malaysia. Med J Malaysia 2003; 58(1): 142-3.
15. Ravindran T, MS, Fatimah S, *et al.* The role of virological surveillance of dengue serotypes for the prediction of dengue outbreak. Trop Biomed 2001; 18(2): 109-16.
16. Sazaly AB, Wong PF. Re-emergence of Dengue 4 virus. Med J Malaysia 2002; 57(2): 242-3.
17. Vinomarlini G, Rogayah T, Saraswathy TS, *et al.* Molecular typing of dengue viruses circulating on the East Coast ofPeninsular Malaysia from 2005 to 2009. Southeast Asian J Trop Med Public Health 2011; 42(1): 94-9.
18. Chee HY, Sazaly AB. Phylogenetic investigation of dengue virus type 2 isolated in Malaysia. Dengue Bulletin 2003; 27: 101-7.
19. Holmes EC, Tio PH, Perera D, *et al.* Importation and co-circulation of multiple serotypes of dengue virus in Sarawak, Malaysia. . Virus Res 2009; 143(1): 1-5.
20. Shamala D. Dengue: Breakbone fever, hemorrhagia or shock. JUMMEC 2008; 11(2):39-52. 21. Jessie K Fong MY, Shamala D, KC Sekaran, *et al.* Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. J Infect Dis 2004; 189(8): 1411-8.
22. MJ. Cardosa. Dengue haemorrhagic fever: questions of pathogenesis. . Curr Opin Infect Dis 2000; 13(5): 471-5.
23. Fong MY, Yusup R, Yusof R, *et al.* Neurovirulence of four encephalitogenic dengue 3 virus strains isolated in Malaysia (1992-1994) is not attributed to their envelope protein. Trans R Soc Trop Med Hyg 2004; 98(6): 379-81
24. Appanna R, Tan LH, Lum LCS, *et al.* Cross-reactive T-cell responses to the nonstructural regions of dengue viruses among dengue fever and dengue hemorrhagic fever patients in Malaysia. Clin Vaccine Immunol 2007; 14(8): 969-77.
25. Appanna R, Wang SM, Ponnampalavanar SA, *et al.* Cytokine factors present in dengue patient sera induces alterations of junctional proteins in human endothelial cells. Am J Trop Med Hyg 2012; 87(5): 936-42.
26. Rathakrishnan A, Wang SM, Hu Y, *et al.* Cytokine expression profile of dengue patients at different phases of illness. PLoS One 2012; 7(12): e52215.
27. Appanna R, Sasheela P, Lum LCS, *et al.* Susceptible and protective HLA class 1 alleles against dengue fever and dengue hemorrhagic fever patients in a Malaysian population. PLoS One 2010; 5(9): 10.1371/journal.pone.0013029.
28. Ooi ET, Ganesananthan S, Anil R, *et al.* Gastrointestinal manifestations of dengue infection in adults. Med J Malaysia 2008; 63(5): 401-5.
29. S. Fadilah, Sahrir S, Mazlam MZ, *et al.* A comparison of the pattern of liver involvement in dengue hemorrhagic fever with classic dengue fever. Southeast Asian J Trop Med Public Health 2000; 31(2): 259-63.
30. Norlijah O, Khamisah A, Kamarul A, *et al.* Serial tourniquet testing in dengue haemorrhagic fever-How clinically useful is it?. Malaysian Journal of Paediatrics and Child Health 2005; 14:27-32.
31. Norlijah O, Khamisah A, Kamarul A, *et al.* Repeated tourniquet testing as a diagnostic tool in dengue infection. Med J Malaysia 2006; 61(1): 22-7.
32. Rusmawati I, Asma Hanim H, Naznin M, *et al.* A descriptive study of blood films of patients serologically positive for dengue in Hospital Tengku Ampuan Afzan, Kuantan. International Medical Journal (IIUM) 2010; 9(2): 35-8.
33. Tong SF, Aziz NA, Chin GL. Prevalence of non-dengue thrombocytopaenia among adult patients presenting with acute febrile illness in primary outpatient clinics. Medicine & health 2006; 1(1): 25-30.
34. Tong SF, Aziz NA, Chin GL, *et al.* Clinical features of acute febrile thrombocytopaenia among patients attending primary care clinics. Malaysian Family Physician 2006; 1(1): 15-8.
35. Tong SF, Aziz NA, Chin GL. Predictive value of thrombocytopaenia in the diagnosis of dengue infection in outpatient settings. Med J Malaysia 2007; 62(5): 390-3.
36. Mustafa B, Hani AW, Chem YK, *et al.* Epidemiological and clinical features of dengue versus other acute febrile illnesses amongst patients seen at government polyclinics. Med J Malaysia 2010; 65(4): 291-6.
37. Norlijah O, Kamarudin NB, Kamarul AR. Clinico-laboratory profile of dengue haemorrhagic fever in Malaysian children. Asian-Oceanian Journal of Pediatrics and Child Health 2004; 3(2).
38. Breen E *et al.*, Early Predictors of Dengue Infection in Adults (EPOD) – A Malaysian Outpatient Experience, Dengue Bulletin 2012, Vol 36.
39. Mohd Zim MA, Sam IC, Omar SF, *et al.* Chikungunya infection in Malaysia: comparison with dengue infection in adults and predictors of persistent arthralgia. J Clin Virol 2013; 56(2): 141-5.

40. Tan PC, Wang SM, Lay KS, *et al.* Dengue infection and miscarriage: a prospective case control study. PLoS Negl Trop Dis 2012; 6(5): e1637.
41. Tan PC, RajasingamG, Devi S, *et al.* Dengue infection in pregnancy: prevalence, vertical transmission, and pregnancy outcome. Obstet Gynecol 2008; 111(5): 1111-7
42. Tee HP, How SH, Jamalludin AR, *et al.* Risk factors associated with development of dengue haemorrhagic fever or dengue shock syndrome in adults in Hospital Tengku Ampuan Afzan Kuantan. Med J Malaysia 2009 64(4): 316-20.
43. Sam SS, Omar SF, Teoh BT, *et al.* Review of dengue hemorrhagic fever fatal cases seen among adults: a retrospective study. PLoS Negl Trop Dis 2013;7(5):e2194.
44. Tan LH, Lum LC, Omar SF, *et al.* Hemophagocytosis in dengue: comprehensive report of six cases. J Clin Virol 2012; 55(1): 79-82.
45. Kamil SM, Mohamad NH, Narazah MY, *et al.* Dengue haemorrhagic fever with unusual prolonged thrombocytopaenia. Singapore Med J 2006; 47(4): 332-4.
46. Rashidi A, Abdul Latiff AK, Salmi AR. Myalgia cruris epidemica: an unusual presentation of dengue fever. Southeast Asian J Trop Med Public Health 2007; 38(6): 1084-7.
47. Umi Kalthum MN, Wong HS. Dengue fever presenting as bilateral dengue maculopathy. Medicine & health 2012; 7(1): 57-61.
48. Tan SY, Kumar G, Surrun SK, *et al.* Dengue maculopathy: a case report. Travel Med Infect Dis 2007; 5(1): 62-3.
49. J M. K. Murthy, Neurological complications of dengue infection. Neurol India 2010; 58: 581-84.
50. Azmin S, S Ramesh, Suehazlyn Z, *et al.* Post-dengue parkinsonism. BMC Infect Dis 2013; 13(179): 2334-13-179.
51. Suhaimi A, Dengue encephalitis. International Medical Journal (IIUM) 2002; 1(2).
52. Masliza M, Noor Darinah MD, Ibtisam M, *et al.* Atrial fibrillation as a complication of dengue hemorrhagic fever: non-self-limiting manifestation. Int J Infect Dis 2009; 13(5): e316-8.
53. Capeding MR, Chua MN, Hadinegoro SR, *et al.* Dengue and Other Common Causes of Acute Febrile Illness in Asia: An Active Surveillance Study in Children. Neglected Trop Dis 2007; 7(7): 1-9.
54. Hamidon BB, Saadiah S. Seoul hantavirus infection mimicking dengue fever. Med J Malaysia 2003; 58(5): 786-7.
55. Yong LS, Koh KC. A case of mixed infections in a patient presenting with acute febrile illness in the tropics. Case Rep Infect Dis 2013; 2013: 562175.
56. Nayar SK, Noridah O, Paranthaman V, *et al.* Co-infection of dengue virus and chikungunya virus in two patients with acute febrile illness. Med J Malaysia 2007; 62(4): 335-6.
57. Ng CF, Lum LC, Noor Azina I, *et al.* Clinicians' diagnostic practice of dengue infections. J Clin Virol 2007; 40(3): 202-6.
58. Lum LC, Goh AY, Chan PW, *et al.* Risk factors for hemorrhage in severe dengue infections. J Pediatr 2002; 140(5): 629-31.
59. Bandyopadhyay S, Lum LC, Kroeger A. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue haemorrhagic fever. Trop Med Int Health 2006; 11(8): 1238-55.
60. Faisal T, Taib MN, Ibrahim F. Reexamination of risk criteria in dengue patients using the self-organizing map. Med Biol Eng Comput 2010; 48(3): 293-301.
61. Faisal T, Ibrahim F, Taib MN. Analysis of significant factors for dengue infection prognosis using the self organizing map. Conf Proc IEEE Eng Med Biol Soc 2008; 2008: 5140-3.
62. Ibrahim F, Ismail NA, Taib MN, *et al.* Modeling of hemoglobin in dengue fever and dengue hemorrhagic fever using bioelectrical impedance. Physiol Meas 2004; 25(3): 607-15.
63. Ibrahim F, Taib MN, Abas WA, *et al.* A novel dengue fever (DF) and dengue haemorrhagic fever (DHF) analysis using artificial neural network (ANN). Comput Methods Programs Biomed 2005; 79(3): 273-81
64. Ibrahim F, Faisal T, Salim MI, *et al.* Non-invasive diagnosis of risk in dengue patients using bioelectrical impedance analysis and artificial neural network. Med Biol Eng Comput 2010; 48(11): 1141-8.
65. Thayan R, Huat TL, Lum LCS, *et al.* The use of two-dimension electrophoresis to identify serum biomarkers from patients with dengue haemorrhagic fever. Trans R Soc Trop Med Hyg 2009; 103(4): 413-9.
66. Thayan R, Huat TL, Lum LCS, *et al.* Differential expression of aldolase, alpha tubulin and thioredoxin peroxidase in peripheral blood mononuclear cells from dengue fever and dengue hemorrhagic fever patients. Southeast Asian J Trop Med Public Health 2009; 40(1): 56-65.
67. Dung N M, Day NP, Tam TH, Loan HT, Chau HTT, Minh LN, Bethell DB, Kneen R, Hien TT, White NJ, Farrar JJ. Fluid replacement in Dengue Shock Syndrome: A Randomized, Double-blind Comparison of Four Intravenous Fluid Regimens. Clinical Infectious Disease 1999; 29: 787-94.
68. Lum LCS, Abdel-Latif Mel-A, Goh AYT, *et al.* Preventive transfusion in Dengue shock syndrome-is it necessary? J Pediatr 2003; 143(5): 682-4.
69. Subenthiran S, Tan CC, Cheong KC, *et al.* Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evid Based Complement Alternat Med 2013; 2013: 616737.
70. Gan CS, Chong SY, Lum LCS, *et al.* Regular paracetamol in severe dengue: a lethal combination? Singapore Med J 2013; 54(2): e35-7.
71. Tan SS, Bujang MA. The clinical features and outcomes of acute liver failure associated with dengue infection in adults: a case series. Braz J Infect Dis 2013; 17(2): 164-9.
72. Lim SV, Rahman MB,, Tejo BA. Structure-based and ligand-based virtual screening of novel methyltransferase inhibitors of the dengue virus. BMC Bioinformatics 2011; 12 Suppl 13: S24,2105-12-S13-S24. Epub 011 Nov 30.
73. Heh CH, Othman R, Buckle MJ, *et al.* Rational discovery of dengue type 2 non-competitive inhibitors. Chem Biol Drug Des 2013; 82(1): 1-11
74. Ang KT, Dengue cluster outbreak in Gombak. Journal of Health Management 2008; 5(2): 55-61.
75. Nasarudin NM, Mohd SI, Ismail AK. The role of an emergency department short-stay ward in the management of dengue fever: a case-control study in a university hospital. Eur J Emerg Med 2013; 20(5): 335-8
76. Sazaly AB, Shafee N. Outlook of dengue in Malaysia: a century later. Malays J Pathol 2002; 24(1): 23-7.
77. Lam SK, Challenges in reducing dengue burden; diagnostics, control measures and vaccines. Expert Rev Vaccines 2013; 12(9): 995-1010.
78. Lam SK, Burke D, Capeding MR, *et al.* Preparing for introduction of a dengue vaccine: recommendations from the 1st Dengue v2V Asia-Pacific Meeting. Vaccine 2011; 29(51): 9417-22.
79. Amar Singh HSS, Koh MT, Tan KK, *et al.* Safety and immunogenicity of a tetravalent dengue vaccine inhealthy children aged 2-11 years in Malaysia: A randomized, placebo-controlled, Phase III study. Vaccine 2013; 31(49): 5814-21.
80. Wong SS, Abd-Jamil J, Sazaly AB. Antibody neutralization and viral virulence in recurring dengue virus type 2 outbreaks. Viral Immunol 2007; 20(3): 359-68.
81. Teoh BT, Sam SS, Juraina AJ, *et al.* Isolation of ancestral sylvatic dengue virus type 1, Malaysia. Emerg Infect Dis 2010; 16(11): 1783-5.
82. Cardosa MJ, Ooi MH, Tio PH, *et al.* Dengue virus serotype 2 from a sylvatic lineage isolated from a patient with dengue hemorrhagic fever. PLoS Negl Trop Dis 2009; 3(4): e423.
83. Norlijah O, Booth A, Intan H, *et al.* Overt bleeding in dengue infection. Asian-Oceanian Journal of Pediatrics and Child Health 2006; 1:23-30.
84. Lum LCS, Suaya JA, Tan LH, *et al.* Quality of life of dengue patients. Am J Trop Med Hyg 2008; 78(6): 862-7.
85. Suaya JA, Shepard DS, Siqueira JB, *et al.* Cost of dengue cases in eight countries in the Americas and Asia: a prospective study. Am J Trop Med Hyg 2009; 80(5): 846-55.
86. Lee HL, Vasan SS, Birgelen L, *et al.* Immediate cost of dengue to Malaysia and Thailand: an estimate. Dengue Bulletin 2010; 34: 65-76.
87. Undurraga EA, Halasa YA, Shepard DS. Use of Expansion Factors to Estimate the Burden of Dengue in Southeast Asia: A Systematic Analysis. PLoS Negl Trop Dis 2013; 7(2): e2056.
88. Shepard DS, Lees RS, Ng CW, Undurraga EA, Halasa Y, Lum LCS. Burden of Dengue in Malaysia. Report from a Collaboration between Universities and the Ministry of Health of Malaysia. 2013.
89. Withehorn J, Van Vinh Chau N, *et al.* Lovastatin for adults patients with dengue: protocol for a randomized controlled trial. Trial 2012 doi: 10.1186/1745-6215-13-203.
90. Capeding M R, Tran N H, *et al.* Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomized, observer-masked, placebo-controlled trial. Lancet 2014 (online publication).