Evaluation of Active RT-PCR (NS1 Ag Assay) Compound for Dengue Haemorrhagic Fever *(Aedes aegypti sp).*

Lalitha S*, Pradeep C and Pavithra R.
Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar,
Salem - 636 011, Tamil Nadu, India

Abstract

The global prevalence of dengue cases has increased in India. The increasingly widespread distribution and the rising incidence of dengue virus infections are related to increased distribution of *Aedes aegypti*, an increasingly urban population, and increasing air travel. Several countries show that the age of the reported dengue cases has increased from 5-12 years, to older children and young adults. Although shock and plasma leakage seem to be more prevalent as age decreases, the frequency of internal hemorrhage rises as age increases. Increase in liver enzymes found in both children and adults indicated liver involvement during dengue infections. Pre-existing liver diseases in adults such as chronic hepatitis, alcoholic cirrhosis, and hemoglobinopathies may aggravate the liver impairment in dengue infection. Fulminant hepatitis is a rare but well described problem in adult patients with dengue infection. Currently, no specific therapeutic agent exists for dengue. The early recognition of dengue infection, bleeding tendency, and signs of circulatory collapse would reduce mortality rates in adult patients with dengue infection.

Keywords: Dengue fever, hemorrhagic fever, plasma, hepatitis, therapeutic.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are re-emerging mosquito-borne viral infections caused by four closely related dengue viruses (serotypes 1-4) of the genus *Flavivirus*. These dengue viruses are by the *Aedes aegypti* and *Ae. albopictus* mosquito species. The dengue virus has antigenically similar but immunologically distinct serotypes. Infection confers lifelong immunity to the infecting serotype therefore; a person can be infected with dengue virus up to four times during his or her lifetime. The World Health Organization (WHO) predicted that there are more than 2.5 billion people living in tropical and subtropical countries, mostly in large and small cities, at risk of dengue infection with one or more dengue viruses.. Ideal conditions for increased transmission of dengue virus in tropical urban centres have been created by substandard housing and crowding, as well as deterioration in water, sewer, and waste management systems, all of which are intimately associated with unplanned urbanization (Barbazan *et al*, 2002; Guzman and Kouri, 2003; Nakhapakorn and Tripathi, 2005; Anyamba *et al*, 2006). Without an effective vaccine or antiviral agent, an effective vector control program is the only means to reduce dengue infection in endemic areas.

The age distribution is different in the places in India, where these syndromes occur in all age groups. Several Asian countries have shown that age of the reported dengue cases has increased from 5-12 years to older children and young adults (Charoensook *et al*, 1999; Pancharoen *et al*, 2002; Pongsumpun *et al*, 2002; Kulanatne *et al*, 2005). The affected adults aged over 15 years old are reported to comprise 20%-40% of dengue virus infected cases according to the Epidemiological Surveillance System (Patumanond *et al*, 2003; Department of Epidemiology, 2012). Morbidity and mortality rates of dengue have been highest in children, especially in the 5-9 year age group. At present, the morbidity rate of DHF has declined to 0.15% while the average age of dengue patients is increasing.

CLINICAL MANIFESTATION

In the early stage of dengue infection, diagnosis from clinical manifestation alone is difficult, especially in adults. Dengue has numerous differential diagnoses, including malaria, leptospirosis, rickettsial diseases, typhoid, chikungunya, other viral hemorrhagic disease, and so forth (Leelarasamee *et al*, 2004; Phuong *et al*, 2006). Dengue infection should be suspected if the patients have a fever of 10 days or less with myalgia,

arthralgia, bone pain, headache, peri-orbital pain, flushing, nausea or vomiting with no obvious respiratory tract symptoms or signs and no organ specific symptoms of other infectious diseases. After an incubation period of 4 to 7 days, the febrile period is accompanied by severe headache, retro-orbital pain, myalgia, arthralgia, nausea, and vomiting. Tantawichien *et al* (2000) described the clinical manifestations of adult patients infected with dengue virus during the epidemic of dengue infection and he reported that there was fever (3 to 8 days), nausea/vomiting, headache, and myalgia in both DF and DHF; however abdominal pain and severe or widespread bleeding manifestations were less frequent in DF.

The tourniquet test has been used as a clue for dengue infection for a long time and has been considered by the WHO in 2009 as one of the criteria for probable dengue infection. Unfortunately, the sensitivity and specificity of tourniquet test from previous report, especially in children, were not excellent, ranging between 34%-56% and 68%-94%, respectively. However, this test was regarded to be a cheap and simple clinical method that is suggestive of dengue when positive, but a negative test does not exclude the disease (Phuong *et al*, 2002; Gregory *et al*, 2011; Mayxay *et al*, 2011; Halsey *et al*, 2013).

LABORATORY TESTS

The test such as reverse transcription polymerase chain reaction (RT-PCR) or dengue non-structural protein 1 antigen, capture assay (NS1 Ag assay) are usually used to diagnose the dengue infection antigen during the early phase of acute infection, and serological ELISA is used to detect specific IgM or IgG antibodies. DF is usually a self-limiting condition, and death as a result is uncommon. Nevertheless, patients who have severe nausea/vomiting, severe hemorrhage (for example, hematemesis, hematochezia, or abnormal vaginal bleeding), hypotension, a platelet count of $\leq 20,000/mm3$ ($\leq 20x109/l$), AST or ALT >500 U/ml, renal failure, liver failure, heart failure, drowsiness, severe hypoxemia, pregnancy, and no opportunity to be followed up in an out-patient setting should be hospitalized. Adults may be at lower risk of developing DHF compared to children and adolescent due to differences in capillary permeability (Gamble et al, 2000). During the transition from the febrile to afebrile phase, DHF patients with increased capillary permeability may manifest with the warning signs, mostly as a result of plasma leakage. The cardinal features that distinguish DHF from DF are increased vascular permeability (plasma leakage syndrome), and marked thrombocytopenia (< 100 x109/l)

associated with bleeding and hepatomegaly and/or abnormal liver function (WHO, 1997).

DIAGNOSIS

Early definite diagnosis of dengue infection can help clinicians in initiation of early supportive care, adequate management, and identification of patients with severe dengue, who should be close monitored for signs of plasma leakage, bleeding, and end organ damage. This information might promote early supportive therapies, prevent the use of potentially harmful drugs, encourage assessment of complications, ensure the adequate use of treatment guidelines, and lead to the effective control of dengue outbreaks. Laboratory diagnosis of dengue infection is established either directly by isolation or detection of viral components in serum or tissue, or indirectly by detection of virus-specific antibodies in human serum (Poerscha *et al*, 2005). The sensitivity of each approach is influenced by the duration and severity of the patient's illness. It should be stressed that in dengue endemic areas, while early accurate laboratory tests are not widely available, dengue infection should be considered in every patient presenting with an acute undifferentiated febrile illness. However, monitoring all these patients for the development of warning signs of severity may impose a great burden on healthcare

services. In the very early stage of illness when patients generally seek medical attention within the first 2-to-3 days of fever without specific symptoms, only RT-PCR or dengue virus NS1 Ag assay can reliably confirm the diagnosis of dengue. RT-PCR is definitely the most satisfactory test that might detect dengue viruses up to the seventh day after the onset of the symptoms, especially in severe cases (Yamada *et al*, 2002; Lanciotti, 2003). In addition, the presence of dengue virus in frozen and fixed tissues, saliva or urine can be determined by RT-PCR. As an early supportive care, adequate management, and identification of patients with severe dengue, who should be closely monitored for signs of plasma leakage, bleeding, and end organ damage. Laboratory diagnosis of dengue infection is established either directly by isolation or detection of viral components in serum or tissue, or indirectly by detection of virus-specific antibodies in human serum (Poerscha *et al*, 2005). The sensitivity of each approach is influenced by the duration and severity of the patient's illness. It should be stressed that in dengue endemic areas, while early accurate laboratory tests are not widely available, dengue infection should be considered in every patient presenting with an acute undifferentiated febrile illness.

However, monitoring all these patients for the development of warning signs of severity may impose a great burden on healthcare services. In the very early stage of illness when patients generally seek medical attention within the first 2-to-3 days of fever without specific symptoms, only RT-PCR or dengue virus NS1 Ag assay can reliably confirm the diagnosis of dengue. RT-PCR is definitely the most satisfactory test that might detect dengue viruses up to the seventh day after the onset of the symptoms, especially in severe cases (Yamada *et al*, 2002; Lanciotti, 2003). In addition, the presence of dengue virus in frozen and fixed tissues, saliva or urine can be determined by RT-PCR.

TREATMENT AND PREVENTION

The early recognition of dengue infection, bleeding tendency, signs of circulatory collapse, and complications would reduce mortality rates in adult patients with dengue infection. Mild dengue infections may be treated at home with oral hydration and antipyretics with instructions to return to the hospital immediately if bleeding or warning signs suggestive of severe disease develop. Oral rehydration is indicated to replace losses from vomiting and high fever. It is necessary to avoid the use of salicylates, NSAIDs, and traditional medicines that may contain hepatotoxic agents. Development of any warning sign

(*eg*, severe vomiting, gastrointestinal haemorrhage, hypotension, high liver transaminase, acute renal impairment, alteration of consciousness, severe thrombocytopenia, etc) indicates the need for hospitalization and close observation with appropriate use of parenteral fluids in patients with inadequate oral intake or a rapidly increasing haematocrit. Attentive clinical monitoring of patients with severe dengue or suspected DHF-DSS and anticipatory and supportive care are life-saving and have reduced fatality rates. WHO recommends immediate volume replacement with Ringer's lactate, or physiologically normal saline solution, followed by fresh frozen plasma or colloid solutions such as albumin, or dextran in the event that shock persists. Crystalloid solutions should be used initially, and isotonic colloid solutions should be reserved for patients presenting with profound shock or those who do not have a response to initial crystalloid therapy. Recently, two randomized controlled trials evaluated therapeutic responses to colloid and crystalloid solutions (Dung *et al*, 1999; Wills *et al*, 2005; Akech *et al*, 2011).

RESULTS

Ringer's lactate performed the least well and that the more severely ill patients identified by a narrow pulse pressure (≤ 10 mmHg) would benefit more from initial

resuscitation with colloid solution than with crystalloid solution. Whole blood, platelet, and fresh-frozen plasma transfusions can be lifesaving for patients with severe bleeding that compromises cardiovascular function, but it should be undertaken with care because of the risk of fluid overload. The use of prophylactic blood or platelet transfusions may be harmful and should be avoided, and invasive procedures should be minimized to avoid hemorrhagic complications. Currently, there is no evidence to support the use of any adjunctive therapies such as corticosteroid, desmopressin, or carbazochrome sodium sulfonate for dengue infection. Dengue prevention currently relies on public health and community-based Aedes aegypti control programs to remove and destroy mosquito-breeding sites. The most advanced approach is a potential vaccine consisting of a tetravalent combination of attenuated dengue strains, and other approaches are undergoing initial clinical evaluation. Dengue will continue to spread worldwide until a safe and effective vaccine is available alongside sustainable mosquito control practices.

REFERENCES

Akech S, Ledermann H, Maitland K. 2011. Choice of fluids for resuscitation in children with severe infection and shock: systematic review. *BMJ*; **341**: c4416.

Alcon S, Talarmin A, *et al*. 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microl* ; **40**; 376-81.

Anyamba A, Chretien JP, Small J, Tucker CJ, Linthicum KJ. 2006-2007. Developing global climate anomalies suggest potential disease risks for. *Int J Health* Geogr 2006; **5**: 60.

Barbazan P, Yokgan S, Gonzalez JP. 2002. Dengue hemorrhagic fever epidemiology in Thailand: description and forecasting of epidemics. *Microb Infect*; **4**: 699-705.

Charoensook O, Foy HM, Teeraratkul A, Silarug N. 1999. Changing epidemiology of dengue hemorrhagic fever in Thailand. *Epidemiol Infect*; **122**: 161-6.

Davis JS, Bourke P. 2004.Rhabdomyolysis associated with dengue virus infection. *Clin Infect Dis;* **38**: e109-11.

Gamble J, Bethell D, Day NP, *et al.* 2000Age-related changes in microvascular permeability: a significant factor in the susceptibility of children to shock? *Clin Sci (Lond); 98*: 211-6.

Gregory CJ, Lorenzi OD, Colo´n L, *et al.* 2011. Utility of the tourniquet test and the white blood cell count to differentiate dengue among acute febrile illnesses in the emergency room. *PLoS Negl Trop Dis*; **5**: e1400.

Guzman MG, Kouri G. 2003. Dengue and dengue hemorrhagic fever in the Americas. *J Clin Virol*; **27**: 1-13.

Halsey ES, Vilcarromero S, Forshey BM, et al. Vol 46 (Supplement 1) 2015 95 Dengue in Adults Performance of the tourniquet test for diagnosing dengue in Peru. Am J Trop Med Hyg 2013; 89: 99-104.

Kalayannarooj S, Vaughn DW, Nimmannitya S, *et al.* 1997.Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis*; **176**: 313-21.

Kittigul L, Pitakarnjanakul P, Sujirara Lanciotti RS 2003. Molecular amplification assays for detection of flaviviruses. *Adv Virus Res*; **61**: 67-99.

Kulanatne SAR, Gawarammana IB, Kumarasiri PRV 2005. Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults. *Southeast Asian J Trop Med Public Health*; **36**: 686-92.

Lee IK, Liu JW, Yang KD. 2005. Clinical characteristics and risk factors for concurrent bacteremia in adults with dengue hemorrhagic fever. *Am J Trop Med Hyg*; **72**: 221-6.

Lanciotti RS. 2003. Molecular amplification assays for detection of *flaviviruses*. *Adv Virus Res* ; **61**: 67-99.

Mayxay M, Phetsouvanh R, Moore CE, *et al.*Massed E, Wilder-Smith A. 2009. Risk estimates of dengue in travelers to dengue endemic areas using mathematical models. *J Travel Med*; **16**: 191-3.

Pancharoen C, Kulwichit W, Tantawichien T,Thisyakorn U, Thisyakorn C. 2002. Dengue infection: a global concern. *J Med Assoc Thai*; **85** (suppl): 25-33.

Patumanond J, Tawichasri C, Nopparat S.2003. Dengue hemorrhagic fever, Uttaradit, Thailand. *Emerg Infect Dis*; **9**: 1348-50.

Phuong HP, Vries PJ, Naga T. 2006. Dengue as a cause of acute undifferentiated fever in Vietnam. *BMC Infect Dis*; **6**:123.

Pongsumpun P, Yokson S, Tang IM. 2002. A comparison of the age distributions in the dengue hemorrhagic fever epidemic in

Santiago de Cuba (1997) and Thailand (1998). *Southeast Asian J Trop Med Public Health*; **33**: 255-8.

Rongrungruang Y, Leelarasamee A. 2001. Characteristics and outcomes of adult patients with symptomatic dengue virus infections. *J Infect Dis Antimicrob Agents*; 18: 19-23.

Schwartz E, Weld LH, Wilder-Smith A, et al. Seasonality, annual trends, and characteristics of dengue among ill returned travelers, 1997-2006. Emerg Infect Dis 2008; 14: 1081-8.

Tantawichien T. 2012. Dengue fever and dengue haemorrhagic fever in adolescents and adults. *Paediatr Int Child Health*; **32** (suppl): 22-7.

Wagner D, With K, Huzly D, *et al.* 2004. Nosocomial acquisition of dengue. *Emerg Infect Dis*; **10**: 1872-3.

WHO,2012. Wung JY, Tseng CC, Lee CS, Cheng KP. 1990. Clinical and upper gastro endoscopic features of patients with dengue virus infection. *J Gastroenterol Hepatol*; **5**: 664-8.

Wills BA, Dung NM, Loan HT, *et al.*2005. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med*; **353**: 877-89.

Yamada KI,Takasaki T, Nawa M, Kurane I. 2002. Virus isolation as one of the diagnostic methods for dengue virus infection. *J Clin Virol*; **24**: 203-9.