ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Serological Analysis of Clinically Diagnosed Dengue Virus Infection by Immunochromatography.

Illamani V[1*], Savetha P[1], Chitralekha S[1], Kiarn M[1], Praveena R[1], Bindu D[2], and Menezes GA[2].

[1]Department of Microbiology, Sree Balaji Medical College and hospital, Chrompet, Chennai, Tamil Nadu, India
[2]Department of Central Research Laboratory, Sree Balaji Medical College and hospital, Chrompet, Chennai, Tamil Nadu, India

ABSTRACT

Dengue is one of the rapidly emerging global threats. Many outbreaks are being noticed nowadays all around the world. In situations of epidemic, early diagnosis is the key to successful management of dengue cases. Many diagnostic kits are available commercially for the same purpose. But their validity is unknown. The gold standard in such situations is IgM capture ELISA, though it is more time consuming. In present study, the test results of one of the commercially available rapid immunochromatographic card test (ICT) were analyzed. Probable dengue cases were diagnosed as per the WHO criteria and the spot test was conducted. A total of 140 probable dengue cases collected over three month's period were included in the study. Of the cases, 17.8% were found to be positive for dengue. In situations of epidemic, the card test can be used for screening dengue cases.

Keywords: Dengue, Immunochromatography, Antibody, NS1 protein, dengue virus.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Dengue viral infection is common mosquito borne febrile illness in developing countries, and is endemic in India. The high prevalence rate and case fatality rate with complications like dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) make early diagnosis in acute phase of disease and management essential [1,2].

Symptomatic Dengue infection patients have a broad spectrum of symptoms which overlap with other viral hemorrhagic fever and bacterial infections. Patients with an acute febrile illness of 2-7 days duration with two or more of the manifestations such as, headache, retro-orbital pain, arthralgia, myalgia, rash, haemorrhagic manifestations, thrombocytopenia, and leucopenia have to be evaluated by laboratory investigation for confirmation of infection. Dengue viraemia in a patient is short, typically occurs 2–3 days prior to the onset of fever and lasts for four to seven days of illness. During this period the dengue virus, its nucleic acid and circulating NS1 viral antigen can be detected. This protein is secreted by virus infected mammalian cells and can be detected as early as day 1 and declines by 5-6 days.[2] Antibody response seen with IgM antibodies which are detectable by 3–5 days after the onset of illness, rise quickly by about two weeks and decline to undetectable levels after 2–3 months. The IgG antibodies are detectable at low level by the end of the first week, increase subsequently and remain for many years. Recently many commercial rapid serological test-kits for detection of NS1 Antigen and anti-dengue IgM and IgG antibodies have become available. They are rapid, easy to perform, less time consuming (results within 10 minutes) and do not require specialised training. They can be done in peripheral setups where the sample load is low and lack of trained staff [3-6].

The objective of the study is serological confirmation of dengue infection in febrile patients who are clinically symptomatic by using ICT (Rapid Diagnostic Tests).

MATERIALS AND METHODS

Study was conducted from routine blood samples collected from patients from Central Laboratory, who are clinically symptomatic with dengue infection at the time of first contact with the hospital and stored with suitable precautions. A total of 140 blood samples were subjected to ICT.

The blood samples from clinically symptomatic patients were collected by venipuncture under sterile precautions, allowed to clot at room temperature (20-25^{o}C) and centrifuged. The serum separated was refrigerated (2-8^{o}C).

Above serum was subjected to rapid ICT using SD BIOLINE Dengue Duo kits. Serum (100µl) was added to sample well and appearance of colored bands at ‘T’ and ‘C’ levels are observed. SD BIOLINE Dengue Duo NS1 Ag + Ab Combo is an ICT designed for the simultaneous detection of NS1 Ag and IgG and IgM antibodies covering all clinical stages of dengue fever infection. If the color band does not appear at ‘C’ (control), the test is considered invalid. Colored bands at ‘C’ and ‘T’ indicates positive test for presence of NS1 Ag and colored bands at ‘C’, ‘M’, ‘G’ indicates IgM and IgG antibodies. The results will be tabulated.

ISSN: 0975-8585

Patients with febrile illness who are clinically symptomatic for dengue infection of all ages and both sex were included in the study. Subjects excluded from the study were, icteric patients and patients with autoimmune diseases. Further, the patients whose serum exhibited hemolysis and was lipaemic was also excluded from the study.

RESULTS

A total of 140 Dengue cases clinically diagnosed during August 2013 to October 2013 were included in the study. The Dengue ICT carried out detected NS1Ag in 17.8% of the total cases. The IgG antibodies were detected in sera of 2.8% subjects, whereas IgM antibodies were present in sera of 1.4% subjects. Interestingly, a serum of a single person (2.1%) was positive for both IgG and IgM antibodies (Table 1).

Table 1: Immunochromatographic card test results obtained during the study period August 2013 to October 2013

Period	Total no of cases	No. of NS1Ag positive No. (%)	No. of IgG Positive No. (%)	No. of IgM Positive No. (%)	No. of both IgG & IgM Positive No. (%)
August 2013	34	6 (17.6%)	-	-	1 (2.9%)
September 2013	51	6 (11.8%)	1 (2.0%)	1 (2.0%)	2 (3.9%)
October 2013	55	13 (23.6%)	3 (5.4%)	1 (1.8%)	-
Total	140	25 (17.8%)	4 (2.8%)	2(1.4%)	3(2.1%)

DISCUSSION AND CONCLUSION

Dengue infection is a rapidly spreading mosquito-borne viral illness caused by flavivirus and transmitted by *Aedes* mosquitoes.[1] The disease is endemic in India with increasing prevalence and has a case fatality rate of 0.65%.

Symptomatic patients have febrile illness and symptoms which overlap with other viral infections such as Chikungunya and Japanese encephalitis. Complications of dengue fever like dengue haemorrhagic fever (DHF), dengue shock syndrome (DSS) which are more common during secondary infection are major international public health concerns. An early and rapid laboratory confirmation of infection is important for the management of the infection [1,2].

Newer rapid diagnostic techniques like ICT, which do not require specialized equipment and training, are being developed. Results will be available from this test within 10 minutes. It can be used for early detection of NS1Ag, along with IgM and IgG antibodies and to differentiate primary and secondary infection [3-6].

In our study, we did not carry out other investigations to compare the results of the ICT test. However, according to the study of Srivatsava *et al,* reported in 2011, out of 91 samples, 26% were positive by NS1 antigen capture ELISA, 16% by SD BIOLINE Dengue NS1 antigen test and 12% positive by RT-PCR analysis. Hence, results of SD BIOLINE Dengue NS1 antigen test correlated well with Panbio NS1 antigen capture ELISA, RT-PCR and virus isolation [7]. Sánchez-Vargas et al., in 2013 reported an overall sensitivity of 90.65% and

specificity of 89.66% for SD BIOLINE Dengue NS1 antigen test [8]. Osorio *et al*., in 2010 found simultaneous detection of NS1/IgM/IgG was potentially useful for dengue diagnosis in both endemic and non-endemic areas [9]. In our study the IgG antibodies were detected in sera of 2.8% subjects, representing convalescent stage of infection, in comparison to IgM antibodies (acute phase) in 1.4% of the subjects.

One more aspect to be noted here was that rapid test used for this study was combination of both antigen {NS1} & antibodies {IgG& IgM}. The positive results were considered irrespective of antigen or antibody. The combination is expected to perform better than isolated detection of antigen and antibody. More studies required to assess the performance of combo test devices and that too on a bigger sample size.

To conclude, in epidemic dengue situations the card test (ICT- SD BIOLINE Dengue) can be used for screening dengue cases. However, a negative result does not rule out dengue case unless a confirmatory test is performed.

REFERENCES

[1] http://apps.searo.who.int/pds_docs/B4751.pdf.
[2] http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf.
[3] Vaughn DW, Nisalak A, Kalayanarooj S, Solomon T, Dung GM, Cuzzubbo A, et al. J Clin Microbiol 1998;36(1):234-8
[4] Sang CT, Hoon LS, Cuzzubbo A and Devine P. Clin Diag Labor Immunol 1998;5(3):407-9
[5] Sharmin R, Tabassum S, Jahan M, Nessa A, Mamun KZ. Dengue Bull 2011;35:84-93
[6] Shankar SG, Dhananjeyan KJ, Paramasivan R, Thenmozhi V, Tyagi BK, Vennison SJ. Clin Microbiol Inf 2012;18(1):8-10.
[7] Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Indian J Med Microbiol 2011;29(1):51-5.
[8] Sánchez-Vargas LA, Sánchez-Marce EE, Vivanco-Cid H. Diag Microbiol Infect Dis. 2014
[9] Osorio L, Ramirez M, Bonelo A, Villar LA, Parra B. Virol J 2010;7:361.