Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Evaluation of NS1Ag and IgM antibodies against dengue, importance for epidemiological surveillance

Nidhi Singla*, Prabhjot Kaur, Jagdish Chander

Department of Microbiology, Government Medical College Hospital, Chandigarh, India

As per the World Health Organization (WHO), a total of 100 tropical and subtropical countries are endemic for dengue, India being one of them[1]. Due to the severe complications involved, non-availability of an effective preventive measure in the form of vaccination and the patient management relying largely on good supportive care, there are enough reasons explaining the importance of early and accurate diagnosis of dengue. The mainstay for diagnosis is serological detection of nonstructural protein 1 (NS1) antigen and antibodies against dengue. However, both the assays have their pros and cons.

NS1 is essential for viral replication hence the amount of secreted NS1 (sNS1) in the serum of patients directly correlates with viremia[2]. NS1Ag has been detected in the blood circulation as early as viral RNA therefore the NS1 Ag assays have the advantage of being positive as early as the first day of fever. Moreover, the detection does not appear to be hindered by the presence of anti-dengue IgM antibodies[3]. NS1Ag assays due to high specificity can also help in serotype determination. Thus for all practical purposes, it is an easy, fast and feasible alternative to RT-PCR in developing countries, for making an early dengue diagnosis[4]. On the other side, overall sensitivity of NS1 Ag detection kit varies widely across the various forms of dengue infection[5], being most sensitive in dengue type 1 infection only. Further, sensitivity is also affected by the day of infection, found to be highest only in patients sampled during the first 9 days after onset of fever[6,7]. It is also less in patients with primary infection and in DF rather than DHF/DSS. The levels vary significantly depending upon the individual affected, phenotype of the virus and storage conditions after sample collection[8].

Considering other option, detecting the antibodies particularly IgM, takes minimum of 10-12 days to come positive and the elevated IgM in a sample could be the result of an infection that occurred 2 to 3 months ago[9,10]. In addition, there is cross reactivity with the infections caused by other flaviviruses including West Nile virus, St. Louis encephalitis virus[11,12], Japanese encephalitis virus and yellow fever virus. Further, IgM levels are significantly lower in secondary dengue infections and thus some false-negative reactions can be observed during secondary infections[13,14]. However, in endemic countries, subclinical infections are common. As a result, patient may fail to present within the first few days of illness (the time period for maximum NS1Ag serologic detection), then, it is the IgM antibody detection which clinches the diagnosis.

In our centre, a total of 844 blood samples were received in the Department of Microbiology from clinically suspected dengue cases from July 2011 to December 2011. The sera were separated and the test was put up for detection of dengue IgM antibodies and NS1Ag by PanBio ELISA, supplied by Inverness Medical Innovations Australia Private Limited, Australia. The test was performed as per the instructions of the manufacturer. Out of a total of 844 samples, a total of 204 (24.17%) samples were positive for dengue virus infection. The month-wise details of these patients are given in Table 1.

A careful evaluation of data shows that if we had performed only dengue IgM ELISA, we would have reported only 157 samples positive for dengue IgM antibodies and would have missed 78 (38%) cases. Similarly, if we had performed only NS1Ag ELISA, we would have reported only 126 samples positive and would have missed 47 (23%) other cases positive for dengue NS1Ag. So, by putting up both the tests

*Corresponding author: Dr. Nidhi Singla, Assistant Professor, Department of Microbiology, Government Medical College Hospital, Chandigarh - 160030, India.
Tel: 0172-2665253-1061
E-mail: nidhisingla76@gmail.com

Table 1
Month-wise details of the cases positive for dengue virus infection.

Month(Total samples positive /Total samples)	OnlyIgM positive/Total IgM positive	Only NS1Ag positive/ Total NS1Ag positive	Both IgM and NS1Ag positive
July 2011 (1/24)	0/1	0/1	1
August 2011(2/60)	1/1	1/1	0
September 2011(16/117)	6/11	5/10	5
October 2011 (120/346)	44/95	25/76	51
November 2011(54/151)	22/ 41	13/32	19
December 2011(11/46)	5/8	3/6	3
Total (204/844)	78/157	47/126	79

on each sample received, we could pick substantial number of additional cases positive for dengue virus infection. If this is the scenario in one institution in one region, considering the whole country/ endemic regions worldwide, the epidemiological significance of this finding can not be ignored. We all are well aware of the importance of determining the full burden of dengue virus infection which can help in guiding public health policies, implementing vector control measures especially household contacts and the need for development of vaccines and antivirals to curtail the disease. Considering the disease a public health priority, to determine the true prevalence of the disease, it is advocated that both assays *ie*. determination of NS1 Ag and IgM antibodies should be performed simultaneously on the given sample.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Kumarasamy V, Chua SK, Hassan Z, Wahab AH, Chem YK, Mohamad M, et al. Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. *Singapore Med J* 2007; **48**: 669-673.

[2] Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* 2002; **186**: 1165-1168.

[3] Xu H, Di B, Pan YX, Qiu LW, Wang YD, Hao W, et al. Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. *J Clin Microbiol* 2006; **44**: 2872-2878.

[4] Bessoff K, Delorey M, Sun W, Hunsperger E. Comparison of two commercially available Dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single clinical sample for diagnosis of acute DENV Infection. *Clin Vacc Immun* 2008; **15**: 1513-1518.

[5] Duong V, Ly S, Lorn Try P, Tuiskunen A, Ong S, Chroeung N, et al. Clinical and virological factors influencing the performance of a NS1 antigen-capture assay and potential use as a marker of dengue disease severity. *PLoS Negl Trop Dis* 2011; **5**: e1244.

[6] Wang SM, Sekaran SD. Early diagnosis of dengue infection using a commercial dengue duo rapid test kit for the detection of NS1, IgM and IgG. *Am J Trop Med Hyg* 2010; **83**: 690-695.

[7] Alcon S , Talarmin A , Debruyne M , Falconar A , Deubel V , Flammand M. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in blood during the acute phase of disease in patient experiencing primary or secondary infection. *J Clin Microbiol* 2002; **40**: 376 - 381.

[8] Alcon-Le P, Sivard SP, Drouet MT, Talarmin A, Rice C, Flamand M. Secretion of flaviviral non-structural protein NS1: from diagnosis to pathogenesis. *Novartis Found Symp* 2006; **277**: 233-47.

[9] Laboratory guidance and diagnostic testing. [Online] Available from: http://www.cdc.gov/dengue/clinicalLab/laboratory.html [Accesed on March 20, 2012].

[10]Wiwanitkit V. Concurrent malaria and dengue infection: a brief summary and comment. *Asian Pac J Trop Biomed* 2011; **1**(4): 326-327.

[11]Bilal H, Hassan SA, Khan IA. Isolation and efficacy of entomopathogenic fungus (Metarhizium anisopliae) for the control of *Aedes albopictus* Skuse larvae: suspected dengue vector in Pakistan. *Asian Pac J Trop Biomed* 2012; **2**(4): 298-300.

[12]Guzmán MG, Kourí G. Dengue diagnosis, advances and challenges. *Int J Infect Dis* 2004; **8**: 69-80.

[13]Suwanbamrung C. Children's basic knowledge and activities for dengue problem solution: an islamic religious school, Southern Thailand. *Asian Pac J Trop Dis* 2012; **2**(6): 456-464.

[14]Vázquez S, Cabezas S, Pérez AB, Pupo M, Ruiz D, Calzada N, et al. Kinetics of antibodies in sera, saliva, and urine samples from adult patients with primary or secondary dengue 3 virus infections. *Int J Infect Dis* 2007; **11**: 256-262.