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Virology

Evaluation of a pan-serotype point-of-care rapid diagnostic assay for accurate detection of acute dengue infection

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ABSTRACT

The catastrophic rise in dengue infections in India and globally has created a need for an accurate, validated low-cost rapid diagnostic test (RDT) for dengue. We prospectively evaluated the diagnostic performance of NS1/IgM RDT (dengue day 1) using 211 samples from a pediatric dengue cohort representing all 4 serotypes in southern India. The dengue-positive panel consisted of 179 dengue real-time polymerase chain reaction (RT-PCR) positive samples from symptomatic children. The dengue-negative panel consisted of 32 samples from dengue-negative febrile children and asymptomatic individuals that were negative for dengue RT-PCR/NS1 enzyme-linked immunosorbent assay/IgM/IgG. NS1/IgM RDT sensitivity was 89.4% and specificity was 93.8%. The NS1/IgM RDT showed high sensitivity throughout the acute phase of illness, in primary and secondary infections, in different severity groups, and detected all 4 dengue serotypes, including coinfections. This NS1/IgM RDT is a useful point-of-care assay for rapid and reliable diagnosis of acute dengue and an excellent surveillance tool in our battle against dengue.

1. Introduction

Dengue is the most common arthropod-borne viral infection transmitted by mosquito to humans that poses a tremendous public health problem. Rapid urbanization, increased travel and climate change have contributed to the increased dengue cases seen globally and in India (Gupta et al., 2012). Dengue virus (DENV) causes an estimated 96 million apparent infections a year globally, and India alone is estimated to contribute 34% of the global total (Bhatt et al., 2013). However, true disease burden based on active surveillance in India is not known. Only a small proportion of dengue-infected symptomatic patients develop severe disease, while the majority recover after a self-limiting illness or go through inapparent or subclinical infection. An early and accurate diagnosis of dengue can lead to early intervention and better clinical management. Moreover, a simple low-cost point-of-care test for dengue surveillance would be useful in assessing the true burden of dengue infection in India.

Understanding the structure of dengue virus and the host immune response has contributed towards development of a range of diagnostic tests. There are four distinct serotypes: DENV1, DENV2, DENV3 and DENV4. All four serotypes have been shown to circulate in India (Chaturvedi and Nagar, 2008; Myers et al., 1970). DENV consists of 3 structural proteins and 7 non-structural proteins. The non-structural protein 1 (NS1) is a glycoprotein released very early from infected cells into blood (Smith and Wright, 1985). Laboratory diagnosis based on viral isolation is highly specific but poor in sensitivity, is labor-intensive and expensive. Use of reverse transcriptase polymerase chain reaction (RT-PCR) may provide an early and accurate diagnosis, but requires specialized equipment not always available in endemic settings. Serological diagnosis using anti-dengue IgM and IgG antibodies is a useful diagnostic and surveillance tool; however, the limitations are its cross-reactivity with other flaviviruses, the need for paired sera for definitive diagnosis, and poor sensitivity during the acute phase of infection when serum antibodies are below the limit of detection (Calisher et al., 1989). Serological assays based on anti-dengue IgM and IgG also have low specificity due to antigenic cross-reactivity and can have decreased utility for early diagnosis of dengue. Dengue NS1 therefore is a useful tool for early diagnosis of dengue and an excellent target for dengue assay development. An NS1 antigen capture enzyme linked immunosorbent assay (ELISA) was developed for the first time in 2000 (Young et al., 2000). Since then, a number of assays have been developed that detect both NS1 antigen and IgM that has increased the diagnostic window period and sensitivity particularly in secondary dengue infection (Blacksell et al., 2008; Fry et al., 2011; Gan et al., 2014). The use of RDTs for point-of-care diagnosis in resource-limited and outbreak

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settings underscores the need for more reliable and accurate NS1 based RDT assays. This study prospectively evaluates the diagnostic performance and operational characteristics of an indigenously developed dengue RDT (Dengue Day 1 Test, J Mitra & Co, New Delhi, India) using clinical samples from a pediatric dengue cohort from southern India. In addition, an NS1 ELISA (DENV Detect™ NS1 ELISA, InBios International, Inc. USA) was also evaluated using the same platform.

2. Methods

2.1. Study setting and population

The current study is part of an ongoing collaborative pediatric dengue cohort study between St John's Medical College Hospital, Bangalore and International Center for Genetic Engineering and Biotechnology (ICGEB), Delhi. Institutional ethical approval was obtained from both institutions prior to patient recruitment. Children with suspected or probable dengue who were admitted to St. John's Medical College Hospital, Bangalore from October 2014 to October 2015 were recruited for the study after obtaining written informed consent. Clinical diagnosis of dengue was made using the WHO guidelines ("World Health Organization and UNICEF, Handbook for clinical management of dengue, 2012"). Patients suspected with acute dengue-like illness and NS1/IgM positive were clinically classified into mild dengue without warning signs (group A), dengue with warning signs (group B) and severe dengue (group C) based on the recent WHO classification("World Health Organization and UNICEF, Handbook for clinical management of dengue, 2012"). Blood samples were collected from each patient on the first day of hospitalization, serum separated for immediate RDT testing and retrospective RT-PCR and anti-dengue IgM/IgG by capture ELISA.

2.2. Laboratory reference tests

The presence of dengue virus and corresponding serotypes was confirmed by RT-PCR and sequencing. Briefly, serum sample from each patient with clinically diagnosed dengue was extracted for total RNA using the QIAamp Viral RNA kit (Qiagen, Germany). Extracted RNA was converted to complementary DNA (cDNA) by reverse transcriptase (Fermentas) using random hexamers (Proteogen). The cDNA was amplified using a sequential algorithm of three modified PCR methods targeting the *capsid premembrane (C-prM)* and *envelope (Env)* regions of the dengue genome in order to maximize DENV detection and serotyping (Ahamed et al., 2016; Lanciotti et al., 1992; Yenchitsomanus et al., 1996). All PCR products were sequenced and nucleotide sequence similarity searches were performed using NCBI BLAST server on GenBank for DENV serotype confirmation. Dengue patients' samples positive by RT-PCR constituted the 'dengue positive panel'. Healthy volunteers and febrile non-dengue patients negative by RT-PCR and negative for anti-dengue IgM and IgG antibodies by capture ELISA (J. Mitra & Co) comprised the 'dengue negative panel'. Primary and secondary infection status of the dengue positive panel was determined using dengue IgM & IgG capture ELISA (PanBio, Product codes 01PE10/01PE20).

2.3. NS1/IgM Rapid Diagnostic Test (RDT)

The Dengue Day 1 Test (J. Mitra & Co) is a rapid solid phase immunochromatographic test with separate cassettes for the qualitative detection of dengue NS1 antigen and differential detection of IgM and IgG antibodies to DENV in human serum. The test was performed according to the manufacturer's instructions. Briefly, 70 μL of serum was added into the sample well of the NS1 cassette and results read at 20 min. To the IgM/IgG antibody cassette, 10 μL of serum was added into the sample well and two drops of dengue antibody assay buffer was added into the buffer well and results read at 20 min. Each cassette has a control and test line; the appearance of both control and test lines indicated a positive result, and appearance of the control line alone indicated a negative result. The test was repeated if the control failed or the result was indeterminate. In this study NS1-only RDT results and combination of NS1 and/or IgM RDT (NS1/IgM RDT) results have been considered for evaluation. IgG RDT results have not been considered, as the presence of IgG antibodies alone may not be used for determining acute dengue infection. The analysis of a single specimen can be completed in about 30 minutes, and a group of 10 specimens within 40 minutes.

2.4. NS1 ELISA

Patients' sera were tested for dengue NS1 antigen using a sandwich immunoassay DENV Detect™ NS1 ELISA (InBios, Seattle, WA, USA) according to manufacturer's instructions. The threshold value of the assay for a positive result was first calculated based on the mean optical density (OD) values obtained with the cut-off controls read at 450 nm. Immune status ratio (ISR) of a sample was then calculated from the ratio of the sample OD divided by the mean OD of the cut-off controls. ISR ≥1 was considered positive for the presence of NS1 antigen. All controls were tested in duplicate, and sera with OD values close to cut-off ($1.1 > ISR > 0.9$) were repeated in duplicate to verify sample status.

2.5. Statistical analysis

The NS1 ELISA, NS1 RDT and NS1/IgM RDT results were compared against the dengue positive panel and dengue negative panel to determine their sensitivity, specificity, positive predictive value and negative predictive value. Sub-group analyses were performed to calculate the sensitivity and specificity of the RDT and ELISA for age, clinical severity, days post-onset of fever, and dengue serotypes. Positive and negative predictive values were calculated based on the prevalence of acute dengue infection in children presenting to the hospital during the study period. Confidence intervals for sensitivity and specificity were set at 95%, and a two-tailed $P < 0.05$ was considered to be statistically significant. All analyses were performed using Stata v13 (Statacorp).

3. Results

A total of 211 samples were included in this study. The dengue positive panel comprised of 179 samples from children with acute symptomatic dengue confirmed by RT-PCR (Fig. 1). The age of the children ranged from 4 months to 15 years (mean age 6.8 ± 4.5 years) and males constituted 61%. Febrile patients presented with a mean 'days post-onset of fever' (DPO) of 4 days (±1.4) and all the dengue positive samples were stratified based on the duration of illness represented by DPO. There were 111 (67.6%) primary and 53 (32.3%), secondary infections within the dengue positive panel (8 samples were equivocal with an IgM/IgG ratio of 1.2 and 7 samples did not have available plasma for serological analyses).

The serotype distribution in the 179 RT-PCR positive samples were as follows; DENV1 (95; 53.1%), DENV2 (46; 25.7%), DENV3 (21; 11.7%), DENV4 (3; 1.7%) and co-infections with two or more serotypes (14; 7.8%). Mild dengue was seen in 2.8%, dengue with warning signs among 57.0%, and severe dengue in 40.2%. The dengue negative panel consisted of 32 samples from febrile and asymptomatic individuals. These included 11 children with febrile non-dengue infection, and 21 healthy volunteers; all these samples were negative for dengue RT-PCR and anti-dengue IgM and IgG by capture ELISA (Fig. 1).

3.1. NS1/IgM RDT

Overall, the NS1/IgM RDT results showed 89.4% sensitivity and 93.8% specificity. The positive predictive value (PPV) and negative predictive value (NPV) was 98.8% and 61.2%, respectively (Table 1). The sensitivity of NS1/IgM RDT in primary infections was 95.4% and in secondary infections was 77.3%.