Section: Microbiology

Original Research Article

COMPARISON OF PERFORMANCE OF IN-HOUSE DUPLEX DENGUE REAL TIME PCR VERSUS CDC ONE-STEP REAL-TIME RT-PCR IN PATIENTS PRESENTING WITH ACUTE UNDIFFERENTIATED ILLNESS

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ABSTRACT

Undifferentiated fever is a common presenting symptom in both adults and children in tropical countries. Despite the control measures, vector-borne diseases contribute to majority of the Acute Febrile Illness (AFI) cases. Complex clinical presentation makes it difficult to distinguish one from another based on clinical history and physical examination alone. Dengue is the fastest spreading vector-borne viral disease. It has been reported from 250 countries and endemic in more than 100 countries including India (Samir Bhatt et al 2013.,) Dengue diagnostics is largely serology-based which includes detection of dengue NS1 antigen, IgM and IgG. Molecular methods like reverse transcriptase-PCR (RT-PCR) are a rapid diagnostic tool but available only in the tertiary care centers. There is a need for the development of indigenous methods that are rapid and cost-effective molecular methods for the detection of dengue viruses as the disease mostly affects people in the lower socio-economic strata.

INTRODUCTION

Acute febrile illness remains the most important public health problem. Tropical climate and limitations in public health issues makes Dengue viruses one of the most important cause for AUFI. Though they present with mild symptoms in majority of the patients. Uncertainty of which patient population will progress to Dengue with warning signs or severe dengue it is important to establish an early diagnosis. Though molecular assays are being used increasing post covid era, it is still not being explored for many other pathogens which we come across in the daily scenarios. Developed countries have standardized assays for outbreak readiness especially for the vector-borne viruses. Dengue viruses 1 to 4 can cause a spectrum of illness ranging from self limiting fever to severe bleeding manifestations. Their detection is largely based on the antigen assay , studies on standardisation of molecular assays are essential to establish molecular assays as point of care testing. We aim to compare the performance of an in-house duplex multiplex RT-PCR with the Multiplex Dengue RT-PCR of CDC , USA.

MATERIALS AND METHODS

The study was conducted in the tertiary care centre at Chennai after obtaining Institutional Ethics committee approval (IEC-NI/12/OCT/30/50).

Study Participants: The study included both in-patients and out-patients attending the medical and pediatrics department in a tertiary care centre at Chennai. Both adult and pediatric patients with acute febrile illness were recruited for the study after obtaining informed consent and assent form respectively fulfilling the following criteria.

Inclusion Criteria

Participants who came to hospital or who were admitted to the medical wards and gave a history of an acute undifferentiated febrile illness (<14 days duration)

Exclusion Criteria

Patients with immunosuppressive conditions other than HIV infection, hematological malignancy, autoimmunedisorders,
immunosuppressivetherapy,and acute infection such as urinary tract infections, bacterial meningitis and suppurative abscesses were not included in the study. Informed Consent was obtained from participants >18 years of age and informed assent were obtained from pediatric participants.

Sample Collection: A total of 150 serum samples were collected (2014-2017) in gel based yellow cap vactuainer tubes (Becton and Dickinson, USA). Clinical details were recorded in specific proforma based on WHO 2009 dengue case definition (Annexure 1). Data on Serological profile of Dengue disease; dengue NS1 antigen (Panbio® Dengue Early ELISA), dengue IgM (Panbio® Dengue IgM Capture ELISA), IgG indicating secondary dengue infection (Panbio® Dengue IgG Indirect ELISA) was documented for all the study participants.

Sample processing and storage: Serum was separated by centrifugation at 1500 rpm for 10 minutes. Samples were aliquoted and immediately stored at -80°C until the RNA extraction

CDC One step real time RT-PCR for detection and serotyping dengue viruses.[1-4]

The CDC Dengue real time RT-PCR was run in a multiplex format for simultaneous detection of DENV1-4. The CDC DENV-1, 2, 3-4 Real-Time RT-PCR assay includes a set of oligonucleotide primers and dual-labelled taqman probes for qualitative detection of DENV serotypes 1, 2, 3 or 4 in serum samples from the study participants (n=150) with acute undifferentiated febrile illness. Targeted regions of viral RNA were transcribed into complementary (cDNA) and amplified by the polymerase chain reaction (PCR). The fluorescently labelled probes annealed to amplified DNA fragments and the fluorescent signal intensity was monitored by the ABI 7500 Fast Dx instrument during each cycle. Any result beyond a cycling threshold of 35 was considered negative.

RNA Extraction: RNA extraction was performed using Qiagen QIAamp® Viral RNA Mini Kit and Invitrogen SuperScript™ III Platinum® was used for the r RT-PCR. Positive control virus mix (heat-inactivated DENV-1 Haw, DENV-2 NGC, DENV-3 H87, and DENV-4 H241) and a Human Specimen Control (HSC) that provides a positive signal in the assay and demonstrates successful recovery of RNA as well as the integrity of the RNA extraction reagent was included in each assay. About (N=100) study participants were tested using the CDC Dengue Real time RT-PCR.

CDC dengue Real time PCR was performed using the reagents provided by the CDC, USA. The assay was performed as per the CDC protocol. About 150 serum samples from the study participants were tested using the CDC Dengue real time serotyping RT-PCR in a multiplex format. Among the 150 study participants tested using the CDC dengue real time RT-PCR of which (n=78 had "probable dengue", n=63 had "dengue with warning signs" and n=9 patients had "severe dengue"). A total of (n= 61) were positive for DENV by CDC Real time dengue PCR, of which n=39 were DENV-1 and n=22 were DENV-2.

DENV-1 was the predominant serotype among patients with warnings signs dengue and severe dengue (Figure 7). PCR positivity was highest among patients with warning signs dengue 83 % (n=30) followed by 80% (n=4) among severe dengue and 38% among with patients with probable dengue as seen in the real time duplex RT-PCR indicating the usefulness of the molecular assays in the 'warning signs dengue group". PCR positivity was highest on day four of the illness(48%).We also found that the CDC dengue PCR was positive even after day 5 of illness in our study participants with "warning signs of dengue".

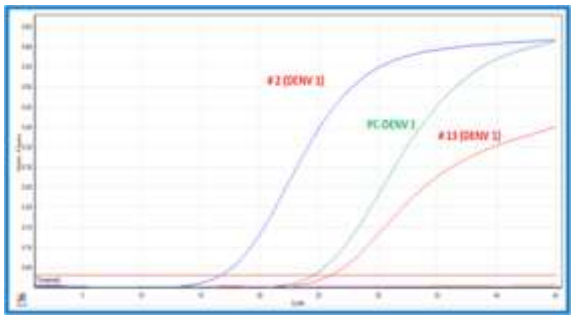

Figure 1: Amplification plot of the CDC dengue real time RT-PCR showing the results of two patients positive for DENV-1.

On comparing the performance of CDC DENV 1-4 real time RT PCR with duplex dengue real time PCR in patients with warnings signs dengue (n=33), the sensitivity was 94.6% Positive predictive value of the CDC real time PCR was 81.4%.

CDC One step real time RT-PCR for detection and serotyping dengue viruses (1-4) The CDC Dengue real time RT-PCR was run in a multiplex format for simultaneous detection of DENV1-4. The CDC DENV-1, 2, 3-4 Real-Time RT- PCR assay includes a set of oligonucleotide primers and dual-labelled TaqMan probes for the qualitative detection of DENV serotypes 1, 2, 3 or 4 in serum samples from the study participants (n=150) with acute undifferentiated febrile illness. Targeted regions of viral RNA were transcribed into complementary (cDNA) and amplified by the polymerase chain reaction (PCR). The fluorescently labelled probes annealed to amplified DNA fragments and the fluorescent signal intensity was monitored by the ABI 7500 Fast Dx instrument during each cycle. Any result beyond a cycling threshold of 35 was considered negative.

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RESULTS

CDC DENGUE REAL TIME RT-PCR CDC dengue Real time PCR was performed using the reagents provided by the CDC, USA. The assay was performed as per the CDC protocol. About 150 serum samples from the study participants were tested using the CDC Dengue real time serotyping RT-PCR in a multiplex format. Among the 150 study participants tested using the CDC dengue real time RT-PCR of which (n=78 had "probable dengue", n=63 had "dengue with warning signs" and n=9 patients had "severe dengue"). A total of (n= 61) were positive for DENV by CDC Real time dengue PCR, of which n=39 were DENV-1 and n=22 were DENV-2.

DENV-1 was the predominant serotype among patients with warnings signs dengue and severe dengue (Figure 5.8). PCR positivity was highest among patients with warning signs dengue 83 % (n=30) followed by 80% (n=4) among severe dengue and 38% among with patients with probable dengue as seen in the real time duplex RT-PCR indicating the usefulness of the molecular assays in the „warning signs dengue group". PCR positivity was highest on day four of the illness(48%).We also found that the CDC dengue PCR was positive even after day 5 of illness in our study participants with "warning signs of dengue". On comparing the performance of CDC DENV 1-4 real time RT PCR with duplex dengue real time PCR in patients with warnings signs dengue (n=33), the sensitivity was 94.6% Positive predictive value of the CDC real time PCR was 81.4%. Figure 1: Interpretation of CDC DENV1-4 real time RT-PCR Figure

Figure 1 Performance of the CDC dengue RT-PCR among the clinical dengue group

DISCUSSION

Dengue remains one of the leading causes of acute undifferentiated febrile illness in tropical and subtropical countries, particularly in India, where all four dengue virus serotypes circulate. Early and accurate diagnosis is essential for appropriate clinical management, timely monitoring of patients at risk of severe disease, and implementation of public health control measures. Although serological assays such as NS1 antigen and IgM antibody detection are widely used, their diagnostic performance varies according to the stage of illness. Molecular methods such as real-time reverse transcriptase polymerase chain reaction (RT-PCR) offer superior specificity and enable early detection of viral RNA before the development of detectable antibodies.

In the present study, the CDC DENV-1–4 real-time RT-PCR assay detected dengue viral RNA in 61 of 150 clinically suspected dengue patients, demonstrating its utility as a reliable molecular diagnostic tool during the acute phase of infection. Among the RT-PCR-positive samples, DENV-1 (39 cases) was the predominant circulating serotype, followed by DENV-2 (22 cases). The predominance of DENV-1 is consistent with reports from several regions of India, although the dominant serotype has been shown to vary geographically and temporally. Continuous serotype surveillance is important because shifts in circulating serotypes have been associated with periodic outbreaks and may influence disease severity in populations with pre-existing immunity.

A notable finding of this study was the higher RT-PCR positivity among patients with dengue with warning signs (83%) and severe dengue (80%) compared with patients classified as probable dengue (38%). This observation suggests that patients with more severe clinical manifestations may have higher or more prolonged levels of viremia, thereby increasing the likelihood of viral RNA detection. Similar observations have been reported in previous studies, where higher viral loads have been associated with increased disease severity, although host immune responses and infecting serotype also contribute significantly to clinical outcomes.

The temporal distribution of RT-PCR positivity demonstrated the highest detection rate on day 4 of illness (48%), which corresponds to the expected period of peak viremia described in previous studies. Importantly, the CDC real-time RT-PCR remained positive beyond day 5 of illness in several patients with dengue with warning signs. This finding suggests that molecular testing may continue to provide diagnostic value beyond the traditionally recognized early viremic phase in patients with more severe disease, possibly reflecting prolonged viral replication or delayed viral clearance. Such observations have important clinical implications, particularly in hospitalized patients presenting later in the course of illness.

The comparison between the CDC DENV-1–4 real-time RT-PCR and the duplex dengue real-time RT-PCR demonstrated excellent diagnostic agreement. The CDC assay showed a sensitivity of 94.6%, indicating its high ability to identify dengue virus infection among clinically significant cases. The positive predictive value of 81.4% further supports its usefulness as a confirmatory molecular diagnostic method. These findings suggest that the duplex real-time RT-PCR performs comparably with the internationally validated CDC assay and has the potential to serve as an indigenous, rapid, and cost-effective alternative for dengue diagnosis in resource-limited settings.

The ability of the assay to simultaneously detect and differentiate dengue serotypes provides an additional advantage for epidemiological surveillance. Early identification of circulating serotypes can facilitate outbreak investigations, monitor changes in serotype distribution, and contribute to understanding the epidemiology of dengue in endemic regions. This is particularly important in countries such as India, where co-circulation of multiple serotypes increases the risk of secondary infections and severe disease.

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The present study has certain limitations. The study was conducted at a single tertiary care center with a relatively modest sample size, which may limit the generalizability of the findings. Viral load quantification was not performed, preventing correlation between viral burden and disease severity. In addition, long-term clinical outcomes were not evaluated. Future multicenter studies involving larger patient populations and quantitative molecular assays would provide further evidence regarding the diagnostic and prognostic utility of real-time RT-PCR.

CONCLUSION

In conclusion, the CDC DENV-1–4 real-time RT-PCR demonstrated excellent diagnostic performance for the detection and serotyping of dengue virus in patients with acute febrile illness. The predominance of DENV-1 observed in this study highlights the importance of continuous molecular surveillance. The high sensitivity of the assay, together with its ability to detect infection in patients with warning signs and even beyond the fifth day of illness, supports the incorporation of molecular diagnostics into routine clinical practice. Furthermore, the comparable performance of the indigenous duplex real-time RT-PCR suggests that it could provide a practical, rapid, and affordable molecular diagnostic alternative for dengue diagnosis in endemic and resource-constrained settings.

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