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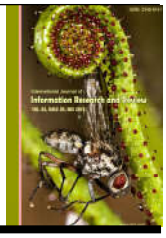

Research Article

DIAGNOSIS AND SEROTYPING OF DENGUE BY RT-PCR

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ABSTRACT

Objective: Dengue is the most important arthropod-borne viral diseases in humans with a large worldwide burden. There are an estimated 50 million infections per year occurring across approximately 100 countries in tropical and sub-tropical regions in the world and potential for further spread.
Methods: Serological and RT-PCR
Results: RT-PCR shown high sensitive with diagnosis of different serotypes as compared to serological tests.
Conclusion: Highly Sensitive and specific diagnosis at species level is prime important for treatment, epidemiological study and control of such infection along with tretment.

INTRODUCTION

Dengue is the most important arthropod-borne viral diseases in humans with a large worldwide burden. There are an estimated 50 million infections per year occurring across approximately 100 countries in tropical and sub-tropical regions in the world and potential for further spread. The disease affects approximately 2.5 billion people living in Southeast Asia, the Pacific, and the Americas (Guzman *et al*., 2010; Simmons *et al*., 2012). Dengue disease causes several clinical manifestations, starting from an undifferentiated fever and dengue fever to the more severe forms of the Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (Simmons *et al*., 2012). Dengue disease is caused by dengue virus (DENV), a member of Flaviviridae family, with a substantial genetic diversity shown by the presence of four serotypes (DENV- 1, -2, -3, and 4) and multiple genotypes (or subtypes) within each serotype (Holmes and Burch, 2000; World Health Organization, 1995). DENV is transmitted through human-mosquito cycle by Aedes aegypti and A. albopictus mosquito vectors. The genome consists of single-stranded positive-sense RNA which encodes three structural (C, prM/M, E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Guzman *et al*., 2010).

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The mortality rate of DHF in most countries is about 5%, primarily among children and young adults. In several Asian countries, it is the leading cause of hospitalization and death in children (5). With the absence of licensed vaccines & specific antiviral therapies for dengue, patient management relies on good supportive care. Prompt and early diagnosis of dengue viral infection remains crucial. Laboratory confirmation is important due to difficulties in making accurate diagnosis due to the broad spectrum of clinical presentations. Among the available dengue diagnostic tools, the detection of virus encoded NS1 antigen has become the basis for commercial diagnostic kits and laboratories are increasingly using NS1 detection as the preferred diagnostic test (Simmons *et al*., 2012). NS1 is a glycoprotein essential for viral replication and viability. Assays have been developed to diagnose DENV infections by detection of NS1 protein in blood during acute phase (Alcon *et al*., 2002). High level early viremia and NS1 antigenemia has also been associated with more severe clinical presentations (Libraty *et al*., 2002).

Dengue IgM and IgG ELISA kits are widely used for diagnosis of dengue infection in routine laboratories. However, there are variations in detection limit during acute phase of the disease. After the onset of symptoms, it usually takes 4–5 and 1–14 days respectively for antiDENV IgM and IgG antibodies to become detectable, depending on whether the patient has primary or secondary infection (Schilling *et al*., 2004).

Isolation of virus in cell culture or in infant mouse brain remains the gold standard for diagnosis of acute cases. However, it requires specialized laboratories and takes more than a week for the test to be completed, making it impractical in most situations. Detection of viral RNA by RT-PCR also allows early diagnosis during febrile phase. However, the procedure is cumbersome and the interpretation is difficult; moreover, the results are not immediate, making its routine use in clinical diagnostic laboratories difficult. In such a state of affairs, there is need for rapid, sensitive and high throughput methods for detection of dengue virus in the early stages of the disease.

MATERIALS AND METHODS

Sample size: patients during the study period 2015-2016 are included. Our tertiary care centre, Wanless Hospital Miraj medical centre Miraj India is a multi-specialty tertiary care centre All hospitalized patients who admitted in ICU, dialysis unit, different wards with High Fever, rash, severe headache and muscle and joint pains are included in this study. Detailed clinical history, laboratory investigations and management of each patient were recorded prospectively and analyzed. A total of 85 Patients K2-EDTA sample was collected and tested for Dengue RTPCR Using Geno-sens kits for amplification &RNA extraction by qigen including Internal , Positive, Negative & standards are included. Same time Dengue Spot & ELISA Serological results are observed. In the present study, we evaluated two new diagnostic tools for acute dengue virus infection. An enzyme immunoassay for detecting dengue virus NS1 antigen in human serum; and a dengue virus specific TaqMan based real time RT-PCR for detection of all four serotypes

RESULTS

As we tested Serologically and RT-PCR for detection of Dengue viral infection almost 50% of patients are shows serologically positive & around 90% patient's RT-PCR for dengue infection was detected along with different serotypes like Dengue 1, 2, 3, and 4. And 10% of blood we failed to extract RNA due to inhibitory products present in Blood. These results shown that RT-PCR shown high sensitive with diagnosis of different serotypes as compared to serological tests.

DISCUSSION

DV-1 was isolated in 1956 at Vellore. All the Indian DV-1 isolates belong to the American African (AMAF) genotype. The Indian DV-1 isolates are distributed into four lineages, India I, II, III and the Africa lineage. Of these, India III is the oldest and extinct lineage; the Afro-India is a transient lineage while India I is imported from Singapore and India II, evolving in situ, are the circulating lineages (Patil *et al*., 2011). The American genotype of DV-2 which circulated predominantly in India during the pre-1971 period was subsequently replaced by the Cosmopolitan genotype. Post-1971 Indian isolates formed a separate subclade within the Cosmopolitan genotype. DV-2 strains were isolated in India over a time span of more than 50 years (1956-2011). The re-emergence of an epidemic strain of DV type-3 in Delhi in 2003 and its persistence in subsequent years marked a changing trend in DV circulation in this part of India (Bhattacharjee *et al*., 1990). Occasional reports of circulation of DV-4 Occasional reports of circulation of DV-4 are also seen, though it is not the predominant type in India (Dar *et al*., 2006; Chaturvedi *et al*., 1970) Laboratory diagnosis of dengue is best made during the acute phase of the illness when DENV circulates in the blood and can be detected by assays to detect the viral RNA genome (Grobusch *et al*., 2006; Johnson *et al*., 2005; Lanciotti, 2003) or soluble antigens (i.e., NS1 antigen) (Peeling *et al*., 2010). Anti-DENV IgM antibody to DENV is also produced during the acute phase of the illness and becomes detectable by ELISA at days 3-5 after onset of fever (Peeling *et al*., 2010; Hunsperger *et al*., 2009). At this point the optimum testing algorithm for dengue has not yet been determined. If results of the DENV detection test (e.g., RT-PCR) are negative (days 1-5 after fever onset), anti-DENV IgM testing should be considered. If the patient first presents during the critical or convalescent phases of the illness, laboratory diagnosis is best made using a test for IgM antibody to DENV.

The performance characteristics of the CDC DENV-1-4 Real-Time RT-PCR Assay were established during a retrospective study at the CDC Dengue Branch. Three hundred seventy one serum samples were obtained from the archived CDC routine dengue surveillance specimens collected in pairs during 2007-2011. The first sample (acute) was collected during the first five days of illness, and the second sample (convalescent) was obtained at least 6 days after the onset of symptoms. These samples were tested with the IgM anti-DENV Capture Enzyme Linked Immunosorbent Assay (CDC MAC-ELISA – validated in-house) in order to establish seroconversion. The results of the CDC DENV-1-4 Real-Time RT-PCR Assay (Multiplex) obtained for the acute samples were compared to the IgM anti-DENV seroconversion results in the paired samples.

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Conflict of Interest

The authors declare that no conflicting interests exist.

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