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COMPARISION OF IGM CAPTURE ELISA WITH A COMMERCIAL RAPID IMMUNOCHROMATOGRAPHIC CARD TEST FOR THE DETECTION OF ANTIBODIES TO DENGUE VIRUSES

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ABSTRACT

Dengue is a major health problem in many parts of the tropical world. It is a mosquito borne illness caused by one of the serotypes of dengue viruses. There is a need for a reliable test for the early diagnosis of dengue fever (DF), which is now active in many parts of India especially in the post monsoon months. This study evaluated two commercial tests with an assay available from a national laboratory in India to obtain information and to make a comparison among the two tests as to which will be the most suited for the detection of IgM antibodies to dengue virus. An IgM capture ELISA (National Institute of Virology, Pune, India) was compared with commercial tests, the Dengue day 1test Rapid Immunochromatographic Card Test (J mitra & co. Pvt. Ltd. New-Delhi) and for the detection of IgM antibodies to dengue virus. We tested 100 samples from individuals with febrile illnesses having DF--like symptoms. The Sensitivity of rapid test (Dengue Day1 Test) with respect to ELISA: 87.5% and Specificity of rapid test (Dengue Day1Test) with respect to ELISA: 97.06% and Positive Predictive value of rapid test (Dengue Day1 Test) with respect to ELISA: 93.33%Platelet count of all the dengue positive cases and healthy controls were recorded and comparison showed that these parameters are statistically significant with p-value <0.0001. These tests should be a useful aid in confirming the clinical diagnosis of dengue infection. The rapid test will be particularly valuable in peripheral health settings, while the ELISA has a place in central testing laboratories.

KEY WORDS: Dengue, ELISA, rapid test, NS-1 antigen

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INTRODUCTION

Dengue is a major health problem in many parts of tropical world[1]. Dengue is caused by an infection with one of the four serotypes of dengue virus (DEN 1- 4) which are Arboviruses belonging to the Flaviviridae family and are transmitted by mosquito principally Aedes aegypti.[2] Dengue is caused by four distinct serotypes of viruses; DEN-1, DEN-2, DEN-3 and DEN-4[3]. Dengue virus causes a spectrum of illness ranging from in apparent, self limiting Classical dengue fever (DF) to life threatening Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). [2] WHO estimates there are 50–100 million dengue infections worldwide every year.[4] The incidence of dengue has grown dramatically around the world over the last 50 years with 2.5 billion people living in areas where dengue is endemic ie over 40% of the world's population – are now at risk from dengue. It affects up to 100 million people each year, with 500,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) who require urgent hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die 6.3 million Children under age five died in 2013, nearly 17 000 die every day Dengue is endemic to the Indian sub-continent.[5] Dengue is associated with explosive urban epidemics and has become a major public health problem in India. It is a notifiable disease, but its exact prevalence is difficult to quantify due to the frequency of epidemics which appear throughout the country.[23] Although dengue serotype 2 is the most prevalent serotype over the past 50 years, serotypes 3 and 4 have appeared in some epidemics.[6-10] Primary DENV infections present as either a non-specific illness or dengue fever (DF). Secondary infection with a serotype different from that causing the primary infection may lead to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).[2] Dengue is an enveloped virus with a single-stranded, positive sense RNA genome of about 11 kb containing a single open reading frame enconding a single polyprotein co- and post translationally cleaved into 3 structural (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS, NS4A, NS4B and NS5)[11] . NS1 is a highly conserved glycoprotein which appears essential for virus viability, although no precise function has yet been assigned to it. During cell infection, NS1 is found associated with intracellular organelles or is alternatively transported through the secretory pathway to the cell surface. A soluble hexameric form may be released in a glycocylation-dependent fashion from infected mammalian cells but not from vector-derived mosquito cells. NS1 is also found circulating during the acute phase of the disease in sera from patients experiencing secondary but not primary infections[12]. The geographical spread of all four DENV serotypes throughout the subtropical regions of the world has led to larger and more severe outbreaks and the accurate and efficient diagnosis of the disease is important for clinical care, surveillance, pathogenesis studies and vaccine research. Furthermore, an efficient diagnosis is an important tool to support Epidemiological Surveillance Programs considering the difficulties in confirming dengue cases based only on the clinical symptoms, especially during inter-epidemic periods.[13] In view of the high mortality rate and to reduce the disease burden, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease. [14] The diagnosis of primary dengue is made by detection of IgM anti-DENV antibodies which appear 5-7 days after the onset of illness and persist for 2-3 months. Secondary infection is characterized by the production of IgG antibodies and a weak IgM response.[15] The major diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR (RT-PCR) & serological tests such as IgM Capture &IgG Capture ELISA. However early dengue diagnosis still remains a major problem as all these assays have their own pitfalls. The first two assays have restricted scope as a routine diagnostic procedure. Viral isolation by Immunoflourescence though a gold standard cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure & time consumption. The MAC- ELISA which is a commonly used assay has low sensitivity in first few days of illness.[14] Now- a-day's detection of NS-1 Ag on rapid tests offer an even faster route to a presumptive dengue diagnosis. NS-1 (Non structural protein) is a highly conserved glycoprotein that is essential for the viability of Dengue virus & is produced both in membrane associated &8 secretary forms by the virus. The detection of secretory NS-1 protein represents a new approach to the diagnosis of dengue infection.[16] Virus isolation and characterization is considered as the gold standard of laboratory diagnosis of acute dengue virus infection. However, it is expensive and atleast 6 to 10 days are required for the virus to replicate in tissue cell culture or laboratory mosquitoes. Reverse transcriptase-polymerase chain reaction (RT-PCR) is also an expensive method and is not widely available in most hospital diagnostic laboratories. A comparison study to evaluate the performance of a commercial rapid dengue immunochromatographic test device (*Acon* Laboratories Inc., USA) for the serological assay of anti-dengue IgM with reference to an IgM-capture Enzyme linked immunosorbent assay.[17] The present study was made to compare the rapid test Dengue day 1test (IgG, IgM, and NS-1 Ag detection) with Capture ELISA (IgG, IgM) ELISA (NIV IgM CAPTURE ELISA manufactured National Institute of Virology, Pune)

DENGUE DAY 1 TEST

Dengue Day 1 Test is a rapid solid phase immuno-chromatographic test for the qualitative detection of Dengue NS1 Antigen and differential detection of IgM and IgG antibodies to Dengue virus in Human serum/plasma. This test is for in vitro diagnostic use only and is intended as an aid in the earlier diagnosis of Dengue infection & presumptive diagnosis between primary and secondary Dengue infection. (J mitra & co. Pvt. Ltd. New-Delhi))

NIV IgM CAPTURE ELISA

DENGUE IgM MICROLISA is designed for in-vitro qualitative detection of Dengue IgM antibodies in human serum or Plasma and is used as a screening test for testing of collected blood samples suspected for DENGUE. The kit detects all four subtypes; DEN1,

DEN2, DEN3 & DEN4 of Dengue Virus. (NIV IgM CAPTURE ELISA manufactured National Institute of Virology, Pune)

MATERIALS AND METHODS

This study was done by prospectively for the year 2014-15 at J.L.N. Medical College and associate group of hospitals, Ajmer. All the suspected dengue cases were first tested by dengue rapid diagnostic test (Dengue day 1test manufactured by J mitra & co. Pvt. Ltd. New-Delhi) for early aid o diagnosis. Then, the results were confirmed by standard ELISA (NIV IgM CAPTURE ELISA manufactured National Institute of Virology, Pune) test by. The dengue suspected case with positive dengue ELISA was considered as confirmed dengue case. The data of confirmed dengue positive case were studied to analysis the epidemiological pattern and clinical features. The data of positive dengue rapid diagnostic test results were compared with the results of dengue ELISA of the same cases[15]

RESULTS

The samples were subjected to Dengue Day 1 rapid test and NIV Dengue IgM Capture ELISA (Table no.1) Out of total 32 cases which were dengue positive by rapid test only 30 were positive by standard dengue ELISA test. Laboratory investigation showed that there was decreased platelet count in DF (Table no.2). 28 cases, among the positive cases of Dengue day 1 test, on further analysis with NIV IgM Capture ELISA were found to be positive, whereas 2 cases among the dengue day 1 test negative, were found positive with NIV IgM capture ELISA.

Table 1

Results of samples (n= 100) tested by Dengue Day 1(IgM) test compared to NIV IgM CAPTURE ELISA

		NIV IgM CAPTURE ELISA	
		Positive(n=30)	Negative(n=70)
Dengue DAY 1 Test(Rapid Anti Dengue IgM)	Positive(n=32)	28	4
	Negative(n=68)	2	66

Sensitivity of rapid test (Dengue Day1 Test) with respect to ELISA: 87.5%
Specificity of rapid test (Dengue Day1 Test) with respect to ELISA: 97.06%
Positive Predictive value of rapid test (Dengue Day1 Test) with respectto ELISA:93.33%

Table 2

Profile of Platelet count

Platelet count(/cu mm)	% of cases
<100000	33
>100000	67

DISCUSSION

Dengue virus is transmitted by mosquitoes of the genus Aedes. Infection with any of the types of dengue virus causes a spectrum of illness ranging from no symptoms or mild fever to severe and fatal hemorrhage and shock depending largely on the patient's age and immunological condition[18]. Transportation, industrialization, movement of infected human population/mosquitoes and the changing ecology have facilitated its spread to newer areas.[19]India is one of the seven identified countries in the South-East Asia region regularly reporting incidence of DF/DHF outbreaks and may soon transform into a major niche for dengue infection in the near future[20]. In our study 33% dengue cases shows <100,000/cu mm & 67% dengue cases shows>1,00,000/cu mm platelet count. These findings do not correlate with study done by R Mehboob[21] which shows only 6% dengue cases with platelet >1,00,000/cu mm & 72% of dengue cases with very low <50,000/cu mm platelet count. Out of 32 rapid dengue diagnostic tests, only 30 were positive by dengue ELISA test. Considering Dengue ELISA as a standard test 94% of the tests were true positive & 06% tests were false positive. Out of total 7 weak positive (light band in rapid diagnostic test), only 02 were positive by standard ELISA test, while 04 were negative by ELISA (false positive). Better sensitivity of IgM-capture ELISA in comparison to rapid ICT have been reported by Moorthy etal and Jayasimha et al (Moorthy M *et al;* 2009; Jayasimha VL *et al ;* 2012). Several other studies showed differences in sensitivity and specificity of ELISA and rapid tests and their difference might be due to the different principles of these assays (Palmer CJ; 1999). Evaluation Series No.3.World Health Organization describes the studies done to evaluate the performance of rapid & ELISA dengue diagnostic tests. Overall, the rapid dengue diagnostic tests showed lower agreement with the reference standard assays for both sensitivity and specificity than the ELISA-based tests. Differences in sensitivity were statistically significant for all comparisons with different company rapid diagnostic tests. Malaria and anti-DENV IgG samples caused the highest false positive IgM rates in both ELISA & rapid.[22]

CONCLUSION

The ELISA test is laboratory gold standard diagnostic test for dengue, but the rapid diagnostic tests are field friendly, with the results available in a short timeframe. Although the rapid diagnostic tests sometimes give false positive results, it can offer early detection of outbreak. Further population based studies are needed, as dengue has emerged as a significant problem in rajasthan, to suggest an effective diagnostic technique for dengue virus detection.

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