

Original article

Detection of Primary and secondary dengue virus infections and its Seroprevalence by immunochromatographic device in tertiary care hospital in central India

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Abstract

Introduction: Dengue is one of the most serious mosquito-borne viral infections affecting tropical and subtropical countries in the world. Since there is no immunoprophylactic or specific antiviral therapy available, timely and rapid diagnosis plays a vital role in patient management and implementation of control measures.

Aims and objectives: The present study was done to know the Seroprevalence of dengue in our region.

Materials & Methods: 270 serum samples were tested in patients clinically suspected Dengue. All the 270samples were subjected to immuno-chromatographic test.

Results: 270 serum samples were tested in patients clinically suspected Dengue. 22 samples were tested positive with ICT (either positive for IgG, IgM or both). Seroprevalence of 8% were reported.

Conclusion: Increase in probable secondary infection seen in this area may lead to DHF epidemics.The commercially available Day 1 dengue rapid test described in the study should be a valuable screening test for dengue fever. It is rapid, easily be performed and interpreted early.

Keyword: Dengue, ELISA, rapid test, Non-Structural-1(NS-1) antigen

Introduction

Dengue is an acute febrile illness endemic to the Indian sub-continent.^[1,2] It is caused by dengue virus (DENV) an arthropod-borne virus of the family *Flaviviridae*. Four distinct serotypes have been described for DENV serotypes 1-4.^[3] The diagnosis of dengue and the differentiation between primary and secondary infections are important not only for monitoring the spread of the epidemic but also for identifying the risk of severe forms of the disease. The detection of immunoglobulin (Ig) M and IgG antibodies is the main technique for the laboratory diagnosis of dengue. Primary DENV infections present as either a non-specific illness or dengue fever (DF). Secondary infection with a serotype different from that causing primary infection may lead to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).^[4] 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.

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.^[5] Viral isolation by Immunofluorescence 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.^[6,7,8]

Nowadays detection of NS-1 Ag on rapid tests offers an even faster route to a presumptive dengue diagnosis. NS-1 (Nonstructural protein) is a highly conserved glycoprotein that is essential for the viability of Dengue virus & is produced both in membrane associated & secretory forms by the virus.^[9] The detection of secretory NS-1 protein represents a new approach to the diagnosis of dengue infection.

Aims and objective

The present study aims to determine primary and secondary dengue infection and its Seroprevalence by immunochromatographic test.

Materials and Methods

The study

The investigations were carried out in central laboratory at Shri Aurobindo medical college and post graduate institute in Indore (M.P.). The study period extended from 1st January to 31st December 2015. The blood was collected from the individual patient, suspected to be suffering from dengue, the serum was separated, and the required test was done on the same date of collection. Serological tests requested by the physicians to diagnose dengue were dengue specific NS1 antigen and IgM and IgG antibodies.

JMitraDengue Day 1 solid phase immunochromatographic test for detection of NS-1 antigen, IgM and IgG antibodies^[10]:

This device is based on the immunochromatographic principle that allows for detection of NS-1, IgG and IgM in a single test. The assay was performed according to manufacturer's instructions and results recorded independently by 2 persons and validated by a supervisor. A positive control was tested along with the samples.

Interpretation of the Dengue Day 1 solid phase immunochromatographic test

The results of the tests are presented as reactive or non-reactive to each of the 3 bands. The reactive results for IgM and/or IgG are more easily found after the viremia that usually disappears by the third day of fever.

status	Result		Interpretation
	IgM	IgG	
1	+	-	recent primary infection
2	+	+	recent secondary infection
3	-	+	secondary infection
4	-	-	no recent infection

Table : 1 Interpretation of serological results for dengue through the dengue rapid test.

IgM: immunoglobulin M; **IgG:** immunoglobulin G; (+): reactive; (-): non-reactive.

Source: Dengue day 1 cassette. Dengue brochure.Cited on 06-2012.

Result

Total of 270 samples were studied. All 270 serum samples were tested for detection of NS1 Ag(antigen), anti-dengue IgM and IgG antibodies by using rapid IC test. Out of 270serum samples 22 (08%) were reactive for NS1 Ag. 14(5%) were non-reactive to NS1 Ag but were seropositive.Total of 22(8%) seropositive cases were detected.

In this 270 samples 08(3%) were reactive to IgM indicating that such patients had dengue for the first time (Primary Dengue infection),10(3.7%) samples were reactive to IgG (Secondary Dengue infection) and 04(1.5%) sample were reactive to both IgM and IgG (recent secondary infection)these patients had dengue for the second or third time (i.e., recent secondary or tertiary infections, which were indistinctly referred to as recent secondary infections).

There were only 14(5%)NS-1 Ag reactive dengue cases.The numbers of secondary dengue cases (IgM & IgG +IgG only = 14) were more than that of primary dengue cases(IgM=8) with a possibility of emergence of DHF or DSS.

Table 2 comparison of test result.

Test result (n=270)	Number	Percentage (%)
1. NS1 antigen reactive only	14	5
2. NS1 antigen &IgM reactive	05	2
3. NS1 antigen &IgG reactive	03	1
4. NS1 antigen non-reactive &IgM+ reactive IgG	04	1.5
5. Only IgM reactive	03	1
6. Only IgG reactive	07	2.5

Discussion

In clinical practice to diagnose dengue serological tests, such as dengue-specific NS1 antigen and IgM and IgG antibodies are now often performed. According to WHO NS1 antigen can be detected up to 9 days after the onset of illness.^[11]

A possible explanation for reduced NS1 Ag in the presence of a measurable anti-DENV antibody response is that the plasma NS1 is sequestered in immune complexes and the target epitopes are not accessible. It is therefore imperative that clinicians and laboratorians should understand the limitations of existing NS1 antigen tests; that a NS1- negative result does not rule out the diagnosis of dengue.^[12]

The assay of anti-dengue specific IgM, depends on the time taken for an infected person's immunological response to produce IgM antibodies against dengue virus antigens. Thus, ICT (often considered as the rapid test for diagnosis of dengue infection) do not provide early diagnosis of acute dengue infection, as in most cases, the first detectable IgM only appears on days 4–5 of the illness.

Increase in probable secondary infection (as evidenced by dual positivity for dengue IgM and IgG) seen in this study is also a point of concern. Such an increase especially in a country like ours where multiple serotypes are prevalent raises concern over probable increase in the incidences of the more serious DHF/DSS.

A few drawbacks of this study are noteworthy:1) The DENV IgM as well as IgG antibodies show some cross reactivity with other members of the *Flaviviridae* family. This can lead to an over estimation of infection rates especially during secondary infection.2) only ICT was used to classify DENV infection in patients. It is likely that some antibody negative samples may have been positive by ELISA, culture or RT-PCR, if it had been attempted.

Conclusion

A pattern developed in which dengue fever epidemic occurred with increasing frequency and were associated with occasional DHF cases. Subsequently, DHF epidemics occurred every few years. Increase in probable secondary infection seen in this area may lead to DHF epidemics.

The commercially available Day 1 dengue rapid test described in the study should be a valuable screening test for dengue fever. It is rapid, easily be performed and interpreted early. However, large studies are needed to determine the most effective way to use them for case management, disease surveillance or whether their present shortcomings can be overcome by a combination testing algorithm.

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