ORIGINAL ARTICLE

Study of Different Serological Markers in Dengue Fever Using Point of Care Rapid Diagnostic Tests

Prasanna Nakate[1], Amit Beldar[2], Yogendra Shelke[1], Suvarna Patil[3] and Sayali Pevekar[1]
[1]Department of Microbiology, [2]Department of Pharmacology, [3] Department of General Medicine
[1,2,3] B.K.L. Walawalkar Rural Medical College, Sawarde-415606,
Maharashtra, India

Abstract:
Background: Dengue is a mosquito borne acute febrile illness with serious complications if not diagnosed and treated early. In many laboratories, it is diagnosed mainly using rapid tests. This study was conducted to know the distribution of different serological markers, in clinically suspected dengue patients using rapid tests. *Material and Methods:* This retrospective study included 2300 serum samples from febrile patients suspected of dengue. The samples were tested for dengue NS-1 antigen, dengue specific IgM and IgG antibodies simultaneously using rapid immuno-chromatographic test. *Results:* Out of 2300 serum samples, 283 were positive for one or more serological parameters of dengue. Seventy two (25.44%) were positive for dengue specific NS1 Antigen, 94 (33.21%) were positive for dengue specific IgM. NS1 antigen and IgM both were positive in 19 (6.71%) samples. In 80 (28.26%) samples both IgM and IgG antibodies were detected. Eighteen (6.36%) samples were positive only for IgG antibody. In primary dengue patients, NS1 antigen alone was positive in 72 (38.92%) cases, IgM positive in 94 (50.81%) and both NS1 antigen & IgM were positive in 19 (10.27%) samples. In secondary dengue infections, IgM & IgG both were detected in 80 (81.63%) samples and IgG alone was found in 18 (18.37%) samples. *Conclusion:* Combined detection of dengue specific NS1 antigen and IgM antibodies is a much sensitive and reliable method of diagnosing primary dengue. Detection of IgG along with clinical evidence of dengue helps to diagnose secondary dengue.

Key words: Dengue, NS1 antigen, IgM antibody, IgG antibody, Primary dengue, Secondary dengue

Introduction:

Acute febrile illness (AFI) constitutes a major portion of patients attending outpatient clinics. One of the commonest causes of AFI in developing countries is dengue fever. It is an important notifiable disease in India and is associated with significant morbidity and mortality if not treated in time. Dengue is a mosquito borne viral disease prevalent all over the world, more so in underdeveloped and developing countries of tropical and subtropical region. Dengue virus belongs to genus Flavivirus and family Flaviviridae. It spreads primarily through the bite of infected *Aedes aegypti* mosquito and to some extent by *Aedes albopictus*.[1] There are four serotypes of dengue virus namely DEN-1, DEN-2, DEN-3 and DEN-4 which are distinct from each other but antigenically related.[2] This accounts for the cross reactivity of antibodies to one serotype with other dengue virus serotypes. If secondary infection occurs with different serotype, then antibodies which were formed against earlier infecting serotype bind to but fail to neutralize the virus. Instead, they protect it from host immune system by inhibiting the bystander B cell activation against the second serotype; a phenomenon call antibody dependant enhancement (ADE). This can lead to potentially fatal complications like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[3] Dengue epidemics are common in south-east Asia including India and have reported case fatality ratio of 3 – 5%.[4] Although there are sophisticated diagnostic procedures such as Real Time PCR (RTPCR) and Enzyme linked Immunosorbent Assay (ELISA) available for dengue, these can be performed only at advanced laboratories. At majority of health care setups, dengue is diagnosed with the help of rapid immunochromatographic tests (ICT) by detecting dengue NS-1 antigen, specific IgM or IgG antibodies either alone or mostly in combination. This study was conducted with an objective of correlating the occurrence of these three serological markers in clinically suspected dengue patients using point of care rapid immunochromatographic tests.

Material and Methods:

This was a retrospective cross sectional observational study of all the consecutive records of patients suffering from acute febrile illness conducted at a tertiary care rural hospital in Konkan region of Maharashtra from January 2018 to December 2020. Serum samples from 2300 patients having an acute febrile illness and clinical features suggestive of dengue were included in this study. Other patients of febrile illness where definitive clinical or laboratory diagnosis was already established were excluded from this study. These samples were tested for the presence of dengue NS-1 antigen and dengue specific IgM and IgG antibodies simultaneously using "Dengue Day 1 test kit" manufactured by J Mitra and Co. Pvt. Ltd, New Delhi, India. It 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. Manufacturer's instructions were strictly followed while performing the tests and analyzing the test results.

Results:

Out of the 2300 serum samples tested, 283 were positive for one or more serological parameters of dengue and 2017 were negative. 12.30% of our patients of acute febrile illness showed dengue seropositivity.

Table – 1: Overall dengue seropositivity in AFI patients

Results	Number of Samples
Dengue Positive	283 (12.30%)
Dengue Negative	2017 (87.69%)
Total	2300

Table – 2: Overall distribution of Serological markers in dengue positive samples

Result of ICT	No. of dengue positive samples
Dengue Specific NS1 Antigen	72 (25.44%)
Dengue Specific IgM	94 (33.21%)
Dengue Specific NS1 & IgM	19 (6.71%)
Dengue Specific IgM & IgG (Both)	80 (28.26%)
Dengue Specific IgG	18 (6.36%)
Total	283

Out of 283 dengue positive samples, 72(25.44%) tested positive for only dengue specific NS1 Antigen, 94 (33.21%) were positive for only dengue specific IgM. Dengue NS1 antigen and IgM antibody both were positive in case of 19 (6.71%) samples with IgG negative. In 80 (28.26%) serum samples both IgM and IgG antibodies were detected. Eighteen (6.36%) serum samples were positive for only dengue specific IgG antibody. Table no. 2 shows the overall distribution of serological markers in dengue positive samples. On the basis of occurrence of different serological markers, we can categorize dengue into primary and secondary types. Presence of Dengue NS1 Antigen or IgM antibody or both indicates primary dengue infection whereas presence of IgG alone or IgM and IgG both indicates secondary dengue infection.[5] Considering this, 185 of our dengue seropositive patients had primary dengue infection with dengue specific NS1 antigen alone positive in 38.92% cases, dengue specific IgM positive in 50.81% and both NS1 antigen & IgM positive in 10.27% samples. In case of remaining 98 cases of secondary dengue infections, dengue specific IgM & IgG both were detected in 81.63% samples and IgG alone was found in 18.37% samples. Table No. 3 depicts distribution of these markers in primary and secondary dengue separately.

Table – 3: Distribution of Serological markers in Primary and Secondary dengue

Type of infection	Result of ICT	No. of positive samples	Total
Primary dengue	Dengue Specific NS1 Antigen	72 (38.92%)	185
	Dengue Specific IgM	94 (50.81%)	
	Dengue Specific NS1 & IgM	19 (10.27%)	
Secondary dengue	Dengue Specific IgM & IgG (Both)	80 (81.63%)	98
	Dengue Specific IgG	18 (18.37%)	

Discussion:

Dengue fever is a common component of differential diagnosis of acute febrile illness especially in tropical

and subtropical regions where it is very common. As its clinical manifestations are similar to other febrile illnesses, the clinical diagnosis of dengue must be confirmed by laboratory methods. Although the PCR based assays for detection of dengue virus or ELISA based techniques for detection of dengue specific antigens or antibodies are more accurate and sensitive, these are not available at every health care set ups. Moreover these methods are more time consuming, need expertise and costly equipment, as compared to the rapid tests. In case of secondary dengue, early diagnosis becomes important as it may lead to severe complications.[5] So rapid immune-chromatographic tests play a very important role in point of care diagnosis of dengue. Here we tried to study the distribution of dengue related serological parameters in patients of fever using these rapid tests. Out of 283 dengue positive samples, 25.44% showed presence of dengue specific NS1 antigen alone without any other marker. Similar findings were reported by Kulkarni RD et al and Rashmi K.S et al where the positivity rate of NS1 antigen among overall dengue patients was 30% and 32.6% respectively.[6, 7] In the present study, NS1 antigen and IgM both were positive in 6.71% cases whereas IgM alone was positive in 33.21% samples. Kulkarni RD et al and Rashmi KS et al reported NS1 antigen and IgM both positive in 11% and 8.7% cases respectively whereas IgM alone positive in 50% and 5% samples respectively.[6,7] In our study, 28.26% samples were positive for both IgM and IgG whereas 6.36% samples were positive for IgG alone. Kulkarni RD and Rashmi K.S et alreported presence of IgM and IgG both in 6% and 21.1% cases respectively whereas IgG alone was reported positive in 3% and 15% respectively.[6,7] In the present study, out of 185 cases of primary dengue infection, 38.92% samples were positive for NS1 antigen alone, 50.81% were positive for dengue specific IgM and 10.27% samples were positive for both NS1 antigen and IgM. This shows that a rapid test with combined detection of NS1 antigen and IgM antibody greatly increases its diagnostic utility in primary dengue than using a test which detects only a single parameter. In our study, out of 98 cases of secondary dengue, 81.63% showed presence of both IgM and IgG, whereas in 18.37% samples only IgG was detected. In general, in dengue virus infections, IgG antibodies develop few days after IgM.[8] These IgG antibodies are serotype specific and may persist for many years after infection. But secondary dengue virus infection generates a strong and quick IgG antibody response and IgG can be detected after 4 – 5 days of illness as compared to 3 – 4 weeks in case of primary infection. In case of acute (early) secondary dengue infection, both IgM and IgG can be detected in serum.[8] One limitation of our study was we did not use ELISA based assays for detection of NS1 antigen, IgM or IgG antibodies which would have been more sensitive than rapid ICT based tests.

Conclusion:

Recently, rapid immunochromatographic tests are playing major role in diagnosis of dengue as they are simple, easy to perform and provide quick results. Combined detection of dengue specific NS 1 antigen and IgM antibodies using a rapid ICT based test is a much sensitive and reliable method of diagnosing primary dengue cases than individually detecting these markers. Though IgG antibodies persist for many years after single dengue infection, detection of IgG along with clinical evidence of dengue fever will definitely help to diagnose secondary dengue cases. Timely detection of cases using point of care rapid tests is as important as other measures such as vector control and management of cases in overall prevention and control of dengue.

Conflicts of Interest: Nil

Source of Support: Nil

References

1. Higa Y. Dengue Vectors and their Spatial Distribution. *Tropical Medicine and Health Supple -ment* 2011; 39(3): 17-27
2. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. *Clinical Microbiology Reviews* 1998; 11(3): 480–496
3. Wen-Hung Wang et al. Dengue hemorrhagic fever - A systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology and Infection* 2020 ; 53:963-978
4. Hartalkar A, Hartalkar S, Wani M, Yadav A. Profile of liver function test in patients with dengue infection. *Walawalkar International Medical Journal* 2015; 2(1): 22–27
5. Changal KH et al. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-

serological study from North India. *BMC Infectious Diseases* 2016;16:2-7

6. Kulkarni RD et al. Association of platelet count and serological markers of dengue infection – Importance of NS1 antigen. *Indian Journal of Medical Microbiology* 2011; 29(4): 359-362
7. Rashmi K.S et al. Serological markers prevalence and trend of probable dengue infection at a tertiary care hospital in Bangalore. *Journal of Evolution of Medical and Dental Sciences* 2013; 2(36): 6968-6976
8. Blacksell SD. Commercial Dengue Rapid Diagnostic Tests for Point-of-Care Application: Recent Evaluations and Future Needs? *Journal of Biomedicine and Biotechnology* 2012; 151967: 1–12 Evaluations and Future Needs? *Journal of Biomedicine and Biotechnology* 2012; 151967:1–12

Received date: 25/04/2023 Revised date: 27/05/2023 Accepted date: 30/05/2023

Address for correspondence:
Dr. Prasanna Nakate,
Professor & HOD of Microbiology
B.K.L. Walawalkar Rural Medical College,
Sawarde , Chiplun, Dist. Ratnagiri, 415606,
Maharashtra, India.
Email: prasannanakate@gmail.com
Mobile: +91 9657743899

How to cite this article: Prasanna Nakate, Amit Beldar, Yogendra Shelke, Suvarna Patil and Sayali Pevekar
Study of Different Serological Markers in Dengue Fever Using Point of Care Rapid Diagnostic Tests
Walawalkar International Medical Journal
2022;09(2):58-61.
http://www.wimjournal.com.