Diagnosis utility of various serological markers including NSI antigen for timely detection of dengue virus infection

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Abstract

Introduction: Fifty to hundred million individuals are believed to be infecting with dengue virus infection worldwide in a year. The dengue virus infected patient presents with varied clinical symptoms. Mostly serological tests are used to detect IgM and IgG antibodies. Few workers also used NS1 antigen for early diagnosis of dengue infection. Therefore, the present study was planned to detect dengue infection timely in the suspected cases by using the various parameters like NS1 antigen, IgM, IgG antibodies along-with platelets.

Materials and Methods: Serum specimens from 777 suspected dengue patients were collected and were analyzed for NS1 antigen, IgM and IgG antibodies using the immunochromatography test kit according to manufacturer's instructions. Platelet counts were also observed for all positive cases.

Results: Out of 777, one hundred ninety-nine (25.6%) patients were found to be positive for either one or more dengue markers (NS1, IgM, IgG). Out of 199 positive specimens, NS1 only, IgM only and IgG only were positive for 128(64.3%), 34(17%) and 3(1.5%) cases respectively. NS1 along with IgM, IgG and IgM+IgG were positive in 12(6.0%), 2(1.0%) and 18(9.0%) cases respectively. Thrombocytopenia was observed in 155(77.8%) dengue positive cases.

Conclusion: Detection of NS1 antigen along with IgM antibodies emerge to be timely, highly specific and reliable for diagnosis of dengue infection. Platelet counts supports as an accessory test for diagnosis of dengue infection.

Keywords: Dengue, IgM antibody, IgG antibody, NS1 antigen, Platelet counts.

Introduction

Dengue infection in humans results from four dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of genus *Flavivirus* and is transmitted to humans by mosquitoes mostly *Aedes aegypti*.[1] WHO (in 1997) classified dengue virus infection into dengue fever, dengue hemorrhagic fever and dengue shock syndrome. In 2009, WHO revised the classification and categorized dengue patients according to different levels of severity as dengue without warning signs, dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets) and severe dengue.[2,3] More than 100 countries, including Europe and the United States, are affected with dengue infection.[4] In India, the 1st case of dengue like illness was reported in Chennai in 1780, and the first virologically proved epidemic of dengue fever occurred in Kolkata and Eastern Coast of India during 1963-1964. The next major outbreak of Dengue/Dengue Hemorrhagic Fever was reported in Delhi and neighboring states in 1996.[5]

Dengue virus infection exhibits varied clinical presentation, therefore, accurate diagnosis is difficult and relies on laboratory confirmation. In most of the cases, serological tests are used to detect IgM and IgG antibodies. Recently, detection of nonstructural protein 1 (NS1) antigen during the acute phase of disease in patients having primary and secondary infections has been studied.[6,7] The non-dengue parameter thrombocytopenia (platelet count<100000/ml) serves as a predictive marker to promote the early diagnosis of dengue infection. Platelet count is the accessory laboratory test available in the peripheral areas that can support the diagnosis of dengue hemorrhagic fever or dengue shock syndrome. Even in remote areas, platelet counts can be roughly estimated by microscopy. Keeping this in mind the present study was planned to detect dengue infection in the suspected cases by using the various parameters like NS1 antigen, IgM, IgG antibodies along-with platelets. A correlation among these parameters was also studied.

Materials and Methods

This prospective study was carried out in the Department of Microbiology, for a period of fifteen months, from September 2016 to November 2017, at a tertiary care hospital. The study received a waiver of informed consent from Institutional Ethical Committee because the samples used were collected for use in approved routine tests. Serum specimens from 777 suspected dengue patients were collected and were analyzed for NS1 antigen, IgM, and IgG antibodies using the immunochromatography test kit according to manufacturer's instructions (J. Mitra and Company Pvt. Ltd. New Delhi, India). The platelet count was also documented in all dengue positive cases.

Results

In the current study, a total of 777 patients were recruited. Out of 777, one hundred ninety-nine (25.6%) patients were found to be positive for either one or more dengue markers (NS1, IgM, IgG). Out of 199 positive specimens, NS1 only, IgM only (Fig. 1) and IgG only were positive for 128(64.3%), 34(17%) and 3(1.5%) cases

respectively. NS1 along with IgM, IgG and IgM+IgG were positive in 12(6.0%), 2(1.0%) and 18(9.0%) cases respectively (Table 1). Platelet count of all positive specimens was recorded. Among 199 positive dengue cases 155(77.8%) patients showed thrombocytopenia (platelet count <100000/ml) (Table 2).

Table 1: Distribution of various positive dengue markers and thrombocytopenia cases

S. No.	Parameters	Positive cases (%)	Thrombocytopenia cases (%)
1	NS1 only	128 (64.3%)	99 (77.3%)
2	IgM only	34 (17%)	25 (73.5%)
3	IgG only	3 (1.5%)	2 (66.7%)
4	NS1 and IgM	12 (6.0%)	12 (100%)
5	NS1 and IgG	2 (1.0%)	2 (100%)
6	NS1, IgM and IgG	18 (9.0%)	13 (72.2%)
7	IgM and IgG	2 (1.0%)	2 (100%)
	Total (n=777)	199	155 (77.8%)

Table 2: Distribution of dengue positive cases according to platelets

Platelets count	Dengue positive (n=199)
<1,00,000 / ml	155 (77.8%)
>1,00,000 / ml	44 (22.2%)

Fig. 1: Antibody IgM only positive among various parameters for detection of dengue infection

Discussion

The incidence of dengue has been increased 30 fold approximate since 1960 to till today. Various factors like rise in population, movement of people from rural to urban, international travel from endemic areas and also global warming may be responsible for this upsurge.[8] Asia and pacific regions where several other infections resembling dengue like malaria, enteric fever are endemic and diagnosis based merely on clinical symptoms is untrustworthy. Therefore, dengue can be easily under diagnosed in the absence of adequate and quality laboratory diagnostic methods. Virus isolation or molecular methods (RT-PCR) is considered as confirmatory tests with high sensitivity and specificity for the diagnosis of dengue infection.[9] However, the need for necessary infrastructure, technical expertise and high cost of the test, make these methods out of reach in a resource poor settings.

Many commercial assays are presently available but in the present study immunochromatograpgy test technique is used for timely detection of dengue infection. Table 1 shows the various dengue specific parameters that were used in this study. In the current study, a total of 199(25.6%) cases out of 777 were positive for either one or more dengue markers (NS1, IgM, IgG). Some workers have reported the varying positivity for either one or more dengue markers. Where Kulkarni et al.[10] showed the 15.2% while Gupta et al.[11] showed the 44.9% seropositivity. Some other authors had shown the different positivity i.e. 39.4% by Tathe et al.[12] and 38.5% by Badave et al.[13] This variability may be due to the different sample size or different region where a particular study has been done.

In the current study, NSI antigen only, in which no antibodies were detected, were positive in 128/199(64.3%) patients. These 128 cases positive for the specific marker NS1 Ag had been missed if NS1 was not recorded. This finding is comparable with studies of Kambale et al.[14] Gupta et al.[11] Tathe et al.[12] and Jyothi et al.[15] that showed the positivity of NS1 antigen only 59.2%, 49%, 60% and 56% respectively.

In the present study out of 199 positive cases 34(17%) and 3(1.5%) are positive for IgM only and IgG only respectively. Some authors have observed varying positivity

for IgM only i.e. 50% by Kulkarni et al.[10] 21.7% by Gupta et al.[11] 4.2% by Kambale et al.[14] and for IgG only Kulkarni et al.[10] observed 3% positivity. NS1 alone or in combination with either IgM or IgG was positive in 160 cases (80.4% cases) in our study that are comparable to the study of Kambale et al.[14] (64.6%), Kulkarni et al.[10] (40.6%) and Badave et al.[13] (71.4%).

In the current study, primary infection (positive for NS1 Ag, IgM, NS1+IgM) was seen in 174 (87.4%) cases and secondary infection (positive for IgG, NS1+IgG, IgM+IgG, NS1+IgM+IgG) was seen in 25 (12.5%) cases. (Table 1) Some workers in their study showed the primary infection 66.7% and secondary 33.3% respectively.[14] Other studies by Golia et al.[16] and Sindhanai et al.[17] reported primary dengue infections 57.4%, 59% and secondary dengue infections 42.6%, 41% respectively.

In the initial phase of infection NS1 antigen is present in the serum that may have undetectable in patients who comes late during the course of infection. NS1 is a highly conserved glycoprotein for all the serotypes and produced in both cell membrane-associated secreted forms.[18,19] It is an early indicator for virus viability or replication especially within the first four days of illness.[20] This supports the fact that a large number of cases would be missed if NS1 was not included in the test panel. When NS1 is positive, there is no need of repeat testing as it is a highly specific marker of dengue infection.[21] Dengue IgM antibodies are usually present following 2-5 days of infection and by combining the results of dengue NS1 antigen and IgM antibody testing, accurate diagnosis during acute presentation is achieved.[22] Between two antibodies, IgG is a less reliable marker in the diagnosis of dengue infection. However, dengue‑specific IgM is a very good indicator of recent infection. It may also be detectable in secondary dengue infection.[21] In Primary dengue case a low titre and slow rising of antibodies will be observed. IgM antibody appears first after 2-5 day followed by IgG antibody at the end of first week of illness. In contrast during secondary infection, rapid increase and high titer of antibodies are seen, i.e., high levels of IgG can be detected even during acute phase of secondary infection and IgM response is variable.[23,24]

In the present study among 199 seropositive cases, thrombocytopenia (<1 lakh) was evident in 155 (77.8%) cases, which is similar to other investigators who reported 68.8% (Kulkarni, et al.[10]), 79.3% (Gupta et al.[11]), 81.1% (Tathe et al.[12]), 60.3% (Badave et al.[13]) and 66.7% (Kambale et al.[14]) thrombocytopenia in positive cases. Thrombocytopenia is the commonest feature in most of the dengue fever cases. In Dengue fever the virus may interact and activate platelets leading to thrombocytopenia.[14] Thrombocytopenia may be related to alterations in megakaryocytopoiesis, manifested by infection of human hematopoietic cells and compromised progenitor cell growth. This may cause platelet dysfunction, damage, or depletion, leading to significant hemorrhages.[9,25] Some workers also observed the cross-reaction between anti-NS1 antibodies with human and mouse platelets that were able to cause transient thrombocytopenia and hemorrhage.[26,27] IL-18 is a type 1 cytokine, produced mainly by monocytes, macrophages, and dendritic cells and high levels of IL-18 may be associated with neutropenia, thrombocytopenia and elevated levels of liver enzymes.[28]

Conclusion

In India, case detection, case management, and vector control are the main tactics for prevention and control of dengue virus transmission. In detection, the present study shows the NS1 antigen along-with IgM antibodies are the earliest detectable marker. The use of this assay can be an effective tool for timely diagnosis and helpful in initiating instant treatment of dengue infection. In case management, at present there is no antiviral therapy is available but the use of supportive therapy with analgesics, sufficient bed rest and hydration with fluid replacement can manage this self-limiting dengue infection. In vector control, by using guppies (Poecilia reticulata) or copepods (doridicola agilis) in stagnant water and infecting the mosquito population with bacteria of the Wolbachia genus the mosquito transmission can be controlled.

Conflicts of Interest: None.

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