ORIGINAL RESEARCH

Detection of NS1 Antigen, IgM and IgG Antibodies Using a Rapid Commercial Dengue Duo Test Kit for the Diagnosis of Dengue Infection at Tertiary Care and Teaching Hospital, GMC Doda J&K

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Received: 21 January, 2023 Accepted: 28 February, 2023

ABSTRACT
Introduction: Dengue is an endemic arboviral disease. Due to the increasing prevalence of dengue infection, early diagnostic confirmation of dengue infection in patients is beneficial for timely clinical intervention, etiological research, and disease management. Evaluation of the Dengue Day 1 test, a commercially available serological test kit. This is for the differential detection of IgM and IgG antibodies in a single acute serum sample intended for detection of Dengue NS1 antigen. **Material and Methods:** The present study was conducted at Department of Microbiology, Govt. Medical College Doda. This study included a total of 700 participants with acute febrile illness. Serum samples were assayed for dengue NS1 Ag, IgM and IgG antibodies using a dengue day 1 assay, a commercially available rapid solid-phase immunochromatographic assay. **Results:** 177 of 700 patients had an acute dengue infection. Of 177, 86 (NS1) tested positive for IgM. Infection rates were higher in the 21-30 age group and men were more affected than women. More dengue cases occurred during and after the rainy season. **Conclusion:** It is recommended that serological tests (NS1Ag, IgM and IgG Ab) are performed early in all cases of suspected dengue so that we can quickly diagnose the disease and initiate the necessary treatment. The current study showed that dengue serological tests play an important role in the early detection of dengue fever.

Keywords: Dengue fever, Dengue hemorrhagic fever, NS1, IgM/IgG antibody

INTRODUCTION

Dengue is a mosquito-borne arboviral disease caused by the genus Flavivirus and transmitted to humans by the bite of infected Aedes mosquitoes, mainly Aedes. Egypt. [1] Dengue infection in humans is caused by four dengue virus serotypes (DEN -1, DEN -2, DEN -3, and DEN -4) of the genus Flavivirus [2]. Dengue virus is a non-segmented, single-stranded, positive-sense enveloped RNA virus; it is transmitted mainly by the bite of Aedes aegypti, a tropical and subtropical mosquito species that lives mainly in urban areas near houses [3]. The WHO (1997) classifies dengue virus infections as dengue fever, dengue hemorrhagic fever, dengue shock syndrome (pain, persistent vomiting, fluid retention, mucosal bleeding, lethargy, liver enlargement, increase in hematocrit with decreasing platelet count), and severe dengue fever [4,5]. Dengue epidemics are becoming more common, especially in the rainy and post-rainy seasons. It can be difficult to diagnose dengue fever in the early stages of the disease because the clinical picture is similar to almost all other viral diseases [6]. Dengue viruses are found in serum or plasma, circulating blood cells, and selected tissues, especially those of the immune system, after the onset of the disease (2 to 7 days), which is approximately the

duration of the fever. [7]. There are three main markers for dengue virus detection, namely H. Dengue NS -1 ICT/ ELISA, dengue serology (IgM & IgG), virus isolation in cell culture, immunofluorescence or detection of viral RNA by nucleic acid amplification tests (NAAT). In acute infection with early symptoms, IgM levels may increase, occurring within 3-5 days and persisting for 30-60 days after infection. In the chronic form of infection, IgG levels may also be elevated 10-14 days after infection and are detectable for life. The hemagglutination inhibition test technique is used [8]. Virus isolation and nucleic acid amplification assays require expertise, expensive equipment and reagents, and time. NS1 antigen and dengue ab(IgM and IgG) detection to detect dengue infection are easy to perform and inexpensive.

In addition, the disease is detected early enough to allow immediate treatment[9,10]. Dengue virus infection has a variable clinical presentation, making accurate diagnosis difficult and dependent on laboratory confirmation. In most cases, serological tests are used to detect IgM and IgG antibodies. Recently, detection of non-structural protein 1 (NS1) antigen during the acute phase of the disease has been investigated in patients with primary and secondary infections. The non-dengue parameter thrombocytopenia can support the diagnosis of dengue hemorrhagic fever or dengue shock syndrome. Even in remote areas (platelet > 1,00,000 /ml), it can be roughly estimated by microscopy. In view of this, the present study was planned to detect dengue infection in suspected cases using various parameters such as NS1 antigen, IgM and IgG antibodies, and platelets, and to investigate a correlation between these parameters [11,12].

MATERIAL AND METHODS

PLACE AND TIME PERIOD OF STUDY

This cross-sectional study was conducted from September to October 2022 in the Department of Microbiology, Govt. Medical College Doda, Jammu and Kashmir.

SAMPLE SIZE

The study included a total of 700 participants with acute febrile illness.

INCLUSION CRITERIA

The demographic profile of each patient was used to determine whether dengue fever was present if two or more of the following symptoms were present: Fever, retro orbital pain, myalgia, arthralgia, rash, nausea/vomiting, and evidence of bleeding.

These blood samples were from patients with acute febrile illness who were hospitalized and treated outside the hospital. To separate the serum, the samples were centrifuged at 2500 rpm for 10 minutes. If processing took longer than expected, serum-free samples were stored in a refrigerator at a temperature of 2° to 8°C. 700 Sera samples were tested for dengue NS1 Ag, IgM, and IgG antibodies (J. Mitra & Co. Pvt Ltd, New Delhi, India). The tests were performed according to the manufacturer's instructions. In brief, the samples and the required amount of Dengue Day 1 test foil bags were warmed to room temperature before testing. Both devices were tested. 2 drops (70µl) of the serum sample were added to the dengue NS1 antigen device and the results were recorded after 20 minutes. For the dengue IgM/IgG device, 10 µl of the sample and 2 drops (70 µl) of the dengue antibody assay buffer were added.

RESULTS

A total of 700 samples were tested and found to be infected with dengue. Of these 700, 177 (25.28%) had serological evidence of dengue disease, and the remaining 532 (76%) were non-dengue patients. Of these 177 seropositive samples, 86 (48.58%) samples were NS1 positive, 32 (18.07%) were both NS1and IgM/IgG Ab positive, and 59 (33.33%) were IgM/IgG Ab positive. Platelet counts were recorded for all positive specimens. Of the 177 positive dengue cases, 126 (71.18%) patients had thrombocytopenia (platelets<100,000/ml) (Table 2).

Table 1: Age-wise distribution of dengue illness (n=700)

Age	No. Pt NS1& IgM/IgG positive	% percent of NS1/%IgM &IgG positive
0-10	117/18	16.71/3.4
11-20	140/28	20/5.35
21-30	169/48	24.14/9.17
31-40	108/29	15.42/5.54
41-50	42/19	6.0/3.63
51-60	72/20	10.28/3.82
>60	52/15	7.42/0.28
Total	700/177	100%

Table 2: Distribution of dengue-positive cases according to platelets (n=177)

Platelets count	Dengue positive (n=177)
<1,00,000/ml	126(71.18%)
>1,00,000/ml	51(28.81%)

Table 3: Sex-wise distribution of patients under study(n=177)

Sex	No.of pt.	%age	NS1 & IgM/IgG	%age
Male	418	59.71	109	61.58%
Female	282	40.28	68	38.41%
Total	**700**		**177**	**25.28%**

Males are more impacted than females, as shown in Table 3 males 109 (61.58%) and female 68 (38.41%) figures.

Table 4: NS1 Ag in dengue patients(n=177)

NS1Ag	No.of Pt.	%age
Only NS1	86	48.58%
NS1with IgM/G	32	18.07%
NS1Negative:IgM/G positive	59	33.33%

DISCUSSION

In tropical countries, it has been observed that most febrile illnesses have similar signs and symptoms, often resembling those of dengue fever, making diagnosis difficult without laboratory confirmation and increasing the burden of the disease [13]. This increase may be caused by several variables, including population growth, a shift from rural to urban areas, international travel from endemic regions, distribution pattern/habitat of vector (*Aedes aegypti*) and global warming [14] Control of dengue infection is challenging because it requires effective vector control. Morbidity and mortality can be prevented by early diagnosis and treatment. Several laboratory methods are available for the diagnosis of dengue infection, such as NS1-Ag, IgM and IgG-Ab, virus isolation, and RNA detection. Therefore, dengue can be easily under diagnosed in the absence of appropriate and high-quality laboratory methods. Virus isolation or molecular methods (RT-PCR) are considered confirmatory tests with high sensitivity and specificity for the diagnosis of dengue infection [9,15]. However, due to the required infrastructure, technical expertise, and high testing costs, these methods are prohibitively expensive in resource-limited settings. Currently, many commercial tests are available, but Immunochromatography was used in the present study, a testing method for the timely detection of dengue infection. A total of 700 samples were tested for NS1 Ag and IgM/G Ab in this study. In current study, dengue cases occurred during the rainy and post-monsoon season, i.e., only from September to November, which is comparable to most previous outbreaks in India [16, 17]. This could be due to the fact that this season is very favorable for the growth of the vector, i.e., *Aedes aegypti*. The difference between serologically positive and serologically negative cases was significantly higher in the post-monsoon season [16,17]. In the study of Ekta et al (2006), most dengue cases occurred in the second and third week of October [18]. This seasonal outbreak of disease transmission is very important at local level for effective control measures. In the present study, most of the patients were predominantly male (418, 61.58%) and less female (282, 38.41%). Our observation was similar and well confirmed by previous studies [19-20]. People of all ages can be infected with dengue virus. In the available studies, the average age group affected ranged from 21 to 30 years. This is consistent with other studies on dengue in India. R.N. Makroo *et al.* [19] In their study, the average age of dengue patients was reported to be 27 years and most of them were in the age group of 21-30 years (24.14%). Ekta Gupta *et al*also reported a preponderance of the age group 21-30 years in their study. We found NS1 positivity in about 86 patients (48.58%) and IgM/G positivity in about 59 (33.33%). This means that the combination of these serological tests would increase the detection rate of dengue fever. These results are similar to those of a study by Fauziah Md *et al.* They found that in 208 cases with suspected dengue fever, NS1 antigen was positive in 67 patients (32.2%) and a total of 107 patients (51.4%) were positive for IgM and IgG antibodies, while combining these tests would increase the detection rate of dengue fever in 129 of 208 patients (62%). Thus, the dengue NS1 antigen test can be used as an adjunct to current antibody detection tests, and the combination of these serological tests would increase the diagnostic efficiency of early detection of dengue disease [21]. Dengue NS1 antigen has attracted considerable interest as a new biomarker for the early detection of dengue infection. NS1 antigen is abundant in the serum of patients in the early stages of infection. It can be detected in peripheral blood before the formation of antibodies from the first day after fever onset until the 9th day after fever onset. Studies have shown that the detection rate of NS1 antigen is higher in acute primary infection than in acute secondary infection [22].

CONCLUSION

In India, case detection, case management and vector control are the most important measures to prevent and control dengue virus transmission. The present study shows that NS1 antigen along with IgM antibodies are the earliest detectable markers. In our study, we found good sensitivity and specificity of the rapid diagnostic tests for early detection of primary dengue infection. These kits are useful for early screening of patients and can further limit the spread

of the disease. These easy- to-perform tests do not require professional or sophisticated equipment and can aid in the early detection of cases and expedite early diagnosis, especially in centers where ELISA capabilities are not available. Studies such as these will therefore make an important contribution to clinical management and may reduce morbidity and mortality from dengue infections.

ABBREVIATIONS

IgM: Immunoglobulin M; IgG: Immunoglobulin G; DENV: Dengue Virus; W.H.O: World Health Organization; DHF: Dengue hemorrhagic fever;

ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to all laboratory techs for assisting in this study.

CONFLICT OF INTERESTS

None

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