Original Article

Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection

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Abstract

Background: The dengue virus causes one of the most important mosquito-borne viral diseases. Annually, it affects up to 100 million people. Detection of the secreted NS1 protein represents a new approach to the diagnosis of acute dengue infection. Platelet count is the only non-dengue parameter that can support the diagnosis of the dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF). This study was done to correlate the platelet count and dengue parameters detected by the immunochromatographic test (ICT).

Materials and Methods: Serum samples collected from patients presenting with dengue-like illness and for whom an anti-dengue antibody test was requested between August 2010 and August 2012, were included in this study. A total of 520 serum samples were collected from the suspected dengue fever patients. The samples were tested for NS1 antigen, IgM, and IgG antibodies, using the ICT kit. The platelet count was recorded in dengue parameter-positive and -negative cases.

Results: A total of 520 serum samples were collected from the suspected dengue fever patients. Sixty-two samples tested positive for one or more dengue-specific parameters. Out of the 62 samples, 39 (62.9%) were positive for the NS1 antigen, only seven (11.3%) were positive for IgM, and only three (4.9%) were positive for IgG. A platelet count < 1,00,000/ml was observed in 32 cases (51.6%). When the platelet count was done in 100 dengue parameter-negative fever patients (controls), thrombocytopenia was observed in 30% of the cases.

Conclusion: Association of thrombocytopenia in dengue parameter-positive cases was highly significant ($Z = 2.76$, $P = 0.006$) when compared to thrombocytopenia in dengue parameter-negative patients.

Key Words: Dengue, NS1 antigen, NS1, thrombocytopenia

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INTRODUCTION

Dengue is an acute febrile illness, endemic to the Indian subcontinent. It is caused by the Dengue virus, and is one of the most significant mosquito-borne viral diseases.[1,2] The Dengue virus (DENV) belongs to the family *Flaviviridae*, and it is transmitted to humans by the *Aedes aegypti* mosquitoes. On the basis of the

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neutralization assay data, four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) can be distinguished. DENV infection is a major cause of disease in tropical and subtropical areas.[3-6], It affects up to 100 million people annually, with 5,00,000 cases of dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), and around 30,000 deaths, mostly among children.[7,8]

Infection with any of the DENV serotypes may be asymptomatic in a majority of the cases or may result in a wide spectrum of clinical symptoms, ranging from a mild flu-like syndrome (known as dengue fever (DF)) to the most severe forms of the disease, which are characterized by coagulopathy, increased vascular fragility, and permeability (DHF). The latter may progress to hypovolemic shock (DSS).[3,9]

Currently the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation, detection of the viral genomic sequence by a nucleic acid amplification technology assay (Reverse transcription polymerase chain reaction (RT-PCR)), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test (ICT).[8,10]

Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage due to its long half-life in blood. The NS1 protein was found to be highly conserved in all dengue serotypes, circulating in high levels during the first few days of illness. It correlates with the development of DHF. There is no cross-reaction of the dengue NS1 protein with those of other related *flaviviruses*.[7,8]

Thrombocytopenia serves as predictive marker to promote the early diagnosis of dengue infection. Apart from the dengue-specific parameters, the platelet count is the only accessory laboratory test available in the peripheral areas that can support the diagnosis of DHF or DSS. Even in remote areas, platelet counts can be roughly estimated by microscopy.[4,11,12] This study was done to correlate platelet count and dengue parameters detected by the immunochromatographic test (ICT) in settings where ELISA and PCR are not available.

MATERIALS AND METHODS

This retrospective study was carried out in the Department of Microbiology, Shri B.M Patil Medical College Hospital, Bijapur, Karnataka, for a period of two years, from August 2010 to July 2012.

Serum samples collected from patients presenting with dengue-like illness and for whom anti-dengue antibody test was requested during the study period, were included in this study. A total of 520 serum samples were collected from the suspected dengue fever patients. The samples were tested for NS1 antigen, IgM, and IgG antibodies using the ICT test kit (Dengue Duo, Dengue NS1 ag + IgG/IgM SD Bioline Standard Diagnostics, INC). The platelet count was recorded in dengue parameter-positive and -negative cases.

Statistical analysis

Statistical analysis was performed with SPSS 14 software. Chi square test and Z tests were applied for analysis of categorical data. A $P < 0.05$ was taken as significant for interpretation.

RESULTS

A total of 520 serum samples were collected from suspected Dengue fever patients. Sixty-two samples tested positive for one or more Dengue-specific parameters [Table 1]. We analyzed the association of thrombocytopenia with dengue parameter positivity [Table 2]. In a total of 62 cases, thrombocytopenia was seen in 32 cases (51.6%). Out of 46 cases that were positive for NS1, thrombocytopenia was observed in 26 cases (56.5%), whereas, when the antibodies alone were considered, thrombocytopenia was observed in six out of 16 cases (37.5%). We found that there was no significant difference (Z = 1.35, P = 0.179) between the above two parameters in relation to thrombocytopenia.

Table 1: Comparison of various dengue parameters

Parameters	Number	Percentage
NS1 only	39	62.9
IgM only	07	11.3
IgG only	03	4.9
NS1 and IgM only	06	9.6
NS1 and IgG only	01	1.7
IgM and IgG only	06	9.6
Total	62	100

IgM: Immunoglobuline M , IgG: Immunoglobuline G, NS1: Non- structural protein 1

Table 2: Comparison of platelet count and dengue parameters

Parameter	Number	Platelet count <1,00,000/ml	Percentage
NS1 only	39	20	51.2
IgM only	07	0	0
IgG only	03	02	66.6
NS1 and IgM only	06	05	83.3
NS1 and IgG only	01	01	100
IgM and IgG only	06	04	66.6
Total	62	32	

IgM: Immunoglobuline M , IgG: Immunoglobuline G, NS1: Non- structural protein 1

Out of 39 cases that were positive for NS1 alone, thrombocytopenia was observed in 20 cases (51.2%), whereas, when NS1 plus IgM antibodies were considered, thrombocytopenia was observed in five out of six cases (83.3%). We found that there was no significant difference ($Z = 1.86$, $P = 0.062$) between the above two parameters in relation to thrombocytopenia.

When the platelet count was completed in 100 dengue parameter-negative fever patients (controls), thrombocytopenia was observed in 30% of the patients. The association of thrombocytopenia in dengue parameter-positive cases was highly significant ($Z = 2.76$, $P = 0.006$), when compared to thrombocytopenia in dengue parameter-negative patients.

DISCUSSION

In order to provide timely information for the management of patients and early public health control of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days of manifestation of the clinical symptoms.[13]

Although virus isolation and characterization are considered as the gold standard for laboratory diagnosis of acute dengue virus infection, it is expensive and takes at least six to ten days for the virus to replicate in a cell culture or laboratory mosquitoes. Detection of the viral genomic sequence by RT-PCR is also an expensive method and is not available in most hospital diagnostic laboratories.[8]

The NS1 antigen is a highly specific marker of dengue infection, as there is no cross-reaction of the dengue NS1 protein, with those of other related flaviviruses. Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage, due to its long half-life in blood.[7,8] The DENV IgM as well as IgG antibodies show some cross-reactivity with other members of the *Flaviviridae* family. This can lead to an overestimation of the infection rates, especially during secondary infection.[1]

In a study conducted by Kulkarni *et al*.,[11] the NS1 alone and with IgM correlated well with thrombocytopenia. In our study, there is no correlation between Dengue seromarkers and thrombocytopenia in Dengue parameter-positive cases. This may be because of the following reasons. The level of NS1 depends on the viral load, because the duration of illness increases, as the level of NS1 decreases. When antibodies start appearing, the NS1 antigen is sequestered into immune complexes.[14] The other reasons for non-correlation are: As ours is a tertiary care center, patients are sent here after few days of treatment in the primary and secondary care centers. More NS1-positive cases would have been detected if the test was done in the first three to four days of fever. Moreover, the sample size in our study is comparatively less.

CONCLUSION

Association of thrombocytopenia in dengue parameter-positive cases was highly significant when compared to thrombocytopenia in dengue parameter-negative cases. In a country like India where most of the hospitals have poor resources, ELISA, viral culture, and PCR cannot be done for the diagnosis of DI, though the sensitivity of these tests is more than ICT. The antibodies take nearly one week to appear in the blood, therefore, antigen detection by the immunochromatographic test is the only means of diagnosis of DI in the first few days of fever, which helps in management of complications like DHF and DSS.

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