ORIGINAL RESEARCH PAPER

Pathology

CORRELATION OF SEROLOGICAL MARKER AND PLATELET COUNT FOR EARLY DENGUE DETECTION IN BUNDELKHAND REGION

KEY WORDS: Dengue, NS1 antigen, NS1, thrombocytopenia.

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ABSTRACT

Background : The dengue virus causes one of the most important mosquito-borne viral diseases and belong to the Flaviviridae family. Dengue infection produces a spectrum of clinical illness, range from asymptomatic to more severe such as dengue shock syndrome and dengue haemorrhagic fever. Detection of the NS1 protein (antigen) is an important marker in the diagnosis of acute dengue fever. Platelet count is only non-dengue parameter which supports the diagnosis of the dengue shock syndrome and dengue haemorrhagic fever.

Aim: The aim of the study was to correlate the platelet count with dengue specific immunological parameters (NS1 antigen & IgM antibody) by the ELISA method.

Material and Methods: Blood sample collected from the patient presenting with dengue like symptoms whom IgM antibody and NS1 antigen test was requested during the period of January 2018 to December 2018 was included in the study. A total 1040 serum samples separated from blood samples. The platelet count was noted in dengue positive and dengue negative cases.

Result: In this study total of 1040 blood samples were collected from suspected Dengue fever patients studied in the Microbiology laboratory from the different wards (IPD) and OPD in the M.L.B. Medical College Jhansi (a tertiary care hospital based study). MLB Medical College, Jhansi is the oldest medical college of Bundelkhand and the demographic data represent whole of this region. One hundred eighty four samples tested positive for one or more Dengue-specific parameters. We analyzed the association of thrombocytopenia with dengue parameter positivity. In a total 184 cases, thrombocytopenia observed in cases 86 (46.7%). Out of 130 that were positive for NS1 antigen, thrombocytopenia was seen in 86 cases (66.1%). We found that there was no significant difference (p= 0.178) between two parameter in relation to thrombocytopenia.

1. INTRODUCTION

Dengue fever is an acute febrile arboviral disease caused by dengue virus belonging to family Flaviviridae and genus Flavivirus affecting tropical & subtropical regions of the world virus transmission in its simplest form involves ingestion of viremic blood by mosquitoes and passage to a second susceptible host [1]. On the basis of the 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 [2, 3]. 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 [4,5].

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 to dengue shock syndrome. Several laboratory methods such as virus isolation, genomic RNA, antigen and antibody detection methods are available to diagnose the dengue infection and methods like virus isolation, genomic RNA detection by PCR, antigen and antibody detection by ELISA needs well trained staff and an expensive setup which is not feasible in all hospital settings [6,7,8].

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 [9, 10]. ELISA directed against NS1 antigen have demonstrated its presence at high concentrations in sera of dengue virus infected patients during early clinical phase of disease [11]. 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 [12, 13, 14].

2. MATERIAL AND METHODS

The study was conducted in the Department of Microbiology M.L.B. Medical College and Hospital from January 2018 to December 2018. A total 1040 serum samples were collected from clinically suspected dengue cases for whom anti-dengue antibody IgM and NS1 antigen test was requested during the study period was including in the study. The samples were tested for NS1 antigen and IgM antibody using enzyme-linked immunosorbent assay (ELISA) Technique. The platelet count was recorded among dengue positive and negative cases.

Detection of NS1 antigen:

ELISA testing for detection of dengue NSI antigen was performed by using commercially available dengue antigen kit as per the manufacturer's instruction (CTK Biotech, Inc. San Diego, CA). The test is based on the principle of solid-phase sandwich ELISA for the qualitative detection of dengue NS1 antigen in serum.

Detection of IgM ELISA:

Dengue IgM ELISA was performed by using "NIV dengue IgM capture ELISA as per as the manufacturer's kit protocol (ICMR-NIV, Pune India). The kit is based on MAC Capture ELISA principle for qualitative detection of IgM antibodies present in human serum

3. RESULT

In this study total of 1040 blood samples were collected that were giving Dengue fever like symptoms received in the Microbiology laboratory from the different wards (IPD) and

OPD in the M.L.B. Medical College Jhansi. One hundred eighty four 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 184 cases, thrombocytopenia observed in cases 86 (46.7%). Out of 130 that were positive for NS1 antigen, thrombocytopenia was seen in 86 cases (66.1%). We found that there was no significant difference (P= 0.178) between two parameter in relation to thrombocytopenia.

Table-1 : Comparison of Dengue parameters.

Parameters	Number	Percentage (%)
NS1Ag only	95	51.6
IgM only	54	29.3
NS1Ag and IgM	35	19.02
Total	**184**	**100**
*Where, IgM: Immunoglobulin IgM, NS1: non structural protein 1		

Table-2 : Comparison of Platelet count and positive case of Dengue parameter.

Parameter	Number	Platelet count <1,00000/ml	Percentage (%)
NS1Ag only	95	40	42.1
IgM only	54	14	25.9
NS1Ag and IgM	35	32	91.4
Total	184	86	
*Where, IgM: Immunoglobulin IgM, NS1: non structural protein 1			

Out of 95 cases that were positive for NS1 antigen, thrombocytopenia was observed in 40 cases (42.1%), whereas, when NS1 antigen with IgM antibodies test were positive, thrombocytopenia was observed in 32 out of 35 cases (91.4%). We observed that there was no significant difference (P = 0.062) between the serological marker of dengue in relation to thrombocytopenia.

When the platelet count was observed in 100 (controls cases) patients suffered from fever and negative for NS1 antigen or IgM antibodies test, thrombocytopenia was observed in 32% of the patients. This association of thrombocytopenia in dengue parameter-positive (NS1Ag/IgM) cases was highly significant (P = 0.006), when compared to thrombocytopenia in dengue parameter-negative (NS1/IgM) patients.

IV 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 [15].

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 flavi viruses. Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage, due to its long half-life in blood [4]. 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.

As the NS1 antigen is detectable in blood from day one after onset of fever, its assay is an effective tool for early diagnosis so as to avoid complications of dengue infection. The ease, speed and dependability of ELISA Method (confirmatory Method) make them an effective technique in addressing this potentially fatal, epidemic prone infection. Platelet count is the only accessory laboratory test available in the remote areas that can support the diagnosis of dengue infection. Therefore studies like this will contribute significantly to the clinical management and can reduce morbidity and mortality in dengue infection [5].

V CONCLUSION

Association of thrombocytopenia in dengue parameter-positive cases was highly significant when compared to thrombocytopenia in dengue parameter-negative cases. In our country most of the Hospital and Laboratories have poor resources, Dengue PCR, Viral Culture, can't be done for dengue infection, though the sensitivity of these tests is more than ELISA. The antibodies take nearly one week to appear in the blood; therefore, antigen detection by the ELISA Method is the only means of diagnosis of dengue infection in the first few days of fever, which helps in management of complications like DHF and DSS. In geographically different areas the endemicity of other diseases causing thrombocytopenia varies. These diseases like malaria and chikungunya must also be ruled out.

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