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A comparative study on Dengue Virus infection: Serological markers Vs Platelet count

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A B S T R A C T

Dengue is an acute infectious disease of viral etiology. Laboratory diagnosis of dengue virus infection mainly depends on detection NS1 antigen of dengue virus and its specific antibodies. Platelet count is the only accessory test for diagnosis of dengue infection in the peripheral laboratories. Therefore, a trial was made to evaluate the association of platelet counts against NS1 and IgM/IgG in dengue infections. Serum samples from clinically suspected dengue cases were tested for NS1, IgM and IgG by rapid test. Platelet counts were obtained for all positive cases and 100 dengue seronegative cases of fever that served as controls. Of 1414 samples tested 281 were found to be positive for the either one or two parameters of dengue serological markers. Platelet count was compared and In cases of fever, thrombocytopenia is more consistently found in dengue positive rather than dengue negative subjects. It correlates well when NS1 and IgM are detected simultaneously.

Introduction

Dengue is an acute febrile illness endemic to the Indian sub-continent.[1] It is caused by dengue virus (DENV) an arthropod-borne virus of the family *Flaviviridae*. Four distinct serotypes have been described for DENV serotypes 1-4.[3] Primary DENV infections present as either a non-specific illness or dengue fever (DF). Secondary infection with a serotype different from that causing primary infection may lead to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).[4] The variability in the clinical illness associated with dengue infection (DI) cannot be accommodated in a single clinical definition. However, confirmation of DI is the most essential prerequisite in the management of complications.[4]

The 'gold standard' tests for identification of DI are not within the reach of peripheral and even most tertiary care laboratories. Detection of dengue specific IgM/IgG has been the mainstay of diagnosis of DI. Antibody detection is an indirect method of diagnosis and, therefore, is prone to false

positive as well as false negative results.[1] Of late, non structural protein 1 (NS1) detection is available for diagnosis of DI.[1] NS1Ag detection is reported to be sensitive as well as highly specific.[4] Apart from the dengue specific parameters, 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. [3]

Materials and Methods

The study was conducted at Rajiv Gandhi Government Women and Children hospital from June 2014 to November 2014 for a period of six months. A total of 1410 samples were collected during the study period from the clinically suspected patients. The test kits used were Advantage dengue NS1 Ag and Ab Combi Card supplied by J Mitra and Co. Pvt. Ltd, New Delhi, India. The tests were performed strictly as per the manufacturer's instructions. Platelet counts of all the cases positive for any of the dengue parameter were recorded. Platelet counts were also recorded in 100 cases of fever that were negative for any of the dengue parameter.

Results and Discussion

Of 1410 samples, 281(19.9%) were found to be positive for either one or more of three markers like NS1Ag, IgM, IgG. Of the 281 serum samples, 177 (62.9%) patients were positive for NS1 only, 74 (26.4%) positive for IgM only, while 16 (5.7%) patients had only IgG. More than one marker was detected in 6 (2.1%) patients were positive for both NS1Ag and IgM, and 8(2.9%) patients were positive for both IgM and IgG. There was no positive samples were found to be positive for NS1Ag and IgG and positive for all the three markers [Table 1]. Platelet count less than 1, 50,000/ml was noticed in 151 cases (50.5%) [Table.1]. Of the 100 cases presenting with fever that were negative for any of the dengue specific parameters by Rapid detection, 30 showed thrombocytopenia (30%).

For a long time detection of dengue specific IgG/IgM has been the mainstay of diagnosis of DI. The dengue specific antibodies begin to appear only around fifth day of fever in primary infection.[2] Even in most secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day.[3] The new parameter, now available, for diagnosis of DI, the NS1 antigen, is detectable from day 1 of fever both in primary and secondary infections. It is important to note that NS1 is shown to be highly specific viral marker making it extremely reliable parameter for the diagnosis of DI from day 1 of the fever.[4]

The various distribution of dengue specific parameters are shown in the Table.1. Of 1414 samples tested 281(62.9%) samples were found to be positive. NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness.[4] NS1 Ag levels varies from 0.04 – 2 μg/ml in acute-phase serum samples, to only 0 .04μg/ml or even less in convalescent phase serum.[6] This is the reason for its higher detection rate in acute phase sera. In our study, NS1 Ag positivity was 62.9% in early primary reactive cases of the dengue infection. The disadvantage of detecting IgM antibodies is being delayed appearance of antibodies from 5-10 days after the onset of illness in case of primary dengue virus infection and 4-5 days after the onset of illness in late primary reactive cases [2]. In our study the percentage of late primary reactive cases of dengue infection is 26.4%, which is almost one half of the early primary reactive cases. Among two antibodies, IgG

is a less reliable marker in the diagnosis of DI.[3] Both clinical and sub clinical infections can produce IgG which may persist for several years affecting the interpretation of test results.[4] It is highly likely that IgG levels could be higher in endemic areas because of bites from infected mosquitoes. In our study IgG positivity was 5.7%, which shows the dengue infection is on late secondary phase. The positivity for the both NS1Ag & IgM is 2.1% which indicates stage of late primary and early secondary reactive cases. The intermediate stage is very less when compared to the other parameters. The positivity for the both antibodies (IgM & IgG) is 2.9%, indicates late secondary reactive cases. This stage of infection is also very less in our study.

We tried to study the comparison of dengue parameter and thrombocytopenia. Out of 281 dengue positive cases, 151 showed the thrombocytopenia. In 177 cases that were positive for NS1, thrombocytopenia was evident in 112 (63.2%) cases. In contrast, when only IgM antibodies were considered for the diagnosis of DI, thrombocytopenia was noted in 30 of 74 (40.5%) cases only. In case of IgG antibodies reactive cases showed 12.5% of thrombocytopenia. While late primary and early secondary reactive cases showed the 33.3% of thrombocytopenia and late secondary reactive showed 12.5% of thrombocytopenia. The control panel of non dengue cases showed 30% of thrombocytopenia. This study shows that the thrombocytopenia is more in the NS1Ag reactive cases i.e., early primary reactive cases of dengue infection when compared to the other parameters.

Thus this study will help in the early diagnosis of the dengue virus infection and the possibility of thrombocytopenia in each of the parameters of the dengue infection.

Table.1 Specific parameters in the diagnosis of dengue infection

Parameters of Dengue infection	**No. of positive cases**	**Percentage of positivity**	**No. of cases to which Platelet count is below 1,50,000/ml**
NS1Ag only	177	62.9	112
IgM only	74	26.4	30
IgG only	16	5.7	2
Both NS1 Ag & IgM	6	2.1	2
Both IgM & IgG	8	2.9	1
Both NS1Ag & IgG	-	-	
All the three parameters	-	-	
Total	281	100	151

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