ORIGINAL RESEARCH

Serological symphony: Harmonizing dengue markers and thrombocytopenia

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ABSTRACT
Dengue fever is the most prevalent arthropod-borne viral infection in human and is classified as major global health threat by the World Health Organisation (WHO).Platelet count is a simple but important accessory test which not only supports the diagnosis of dengue and also helps to monitor the disease progression.Platelet count of dengue positive cases were significantly lower than the dengue negative cases. While correlating dengue parameters with platelet count, thrombocytopenia was common in NS1 antigen associated cases than with cases positive for antibodies alone. Thrombocytopenia was much observed in secondary dengue infections than the primary infections. **Aims & Objective**:To evaluate diagnostic parameters inDengue febrile patients, who are ELISAconfirmed and tocorrelate the clinical condition with platelets count. **Methodology:** This prospective study conducted for a period of one year in Department of Microbiology, Gajra Raja Medical Collegeand J. A. Group of Hospitals (JAH), Gwalior, Madhya Pradesh.This study was performed among patients with febrile illness who were clinically symptomatic for dengue infection. A structured assessment form was used to obtain the clinical history regarding febrile illness including clinical symptoms and signs. Blood samples from clinically symptomatic OPD and IPD patients were collected. **Results:** A total number of 1,762 samples of clinically suspected dengue fever was processed for serological markers of dengue during the period of one year from January 2019 to December 2019.Among 1,762 samples,out of the 568 total positive cases of dengue, 439 (77.29%) showed thrombocytopenia. In 249 cases that were positive for NS1 Ag, thrombocytopenia was evident in 197 (79.12%) cases. and when IgM antibodies is found to be positive thrombocytopenia was noted in 151 out of 211 (71.56%) cases. when both the markers NS1 Ag + IgMAb was positive, thrombocytopenia was observed in 91 out of 108 (84.25%) cases. **Conclusion**: Currently dengue is globally the most important and rapidly spreading mosquito-borne viral disease.Out of these dengue specific parameters, platelet count is the only laboratory parameter performed in remote areas because of cost effectiveness and easy to perform that can support the diagnosis of dengue infection. Such predictions will help to decrease complication due to late treatment and initiate the preventive and control measures well in time for the containment of spread of the disease and contribute significantly to the clinical management which reduce morbidity and mortality in dengue infection.
Key words: Dengue, Seropositivity, Serological markers,Thrombocytopenia, platelet count.

INTRODUCTION

Dengue is presently regarded globallyas the most important and rapidly spreading mosquito-borne viral disease.It is a cause of great concern to public health in India. Every year,thousands of peoples are affected and contribute to the burden of health care.[1,2]The etiological agent of disease is dengue virus (DENV), a member of the family *Flaviviridae*. It affects 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.[3,4,5]

The combined case fatality rate is around 5% for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[6]It is known thatearly and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality.[7,8]

The ‘gold standard’ tests for the identification of dengue infection (DI) are not in the reach of peripheral and even most tertiary care laboratories.

Serological diagnosis by detection of antibodies is widely used, but antibodies appear only after 4 to 6 days of illness.[9] Secretory protein NS1 antigen is seen in high concentrations during acute phase of illness (1 to 5 days).[10]Combination of NS1 antigen detection along withantibody detection increases the diagnostic rates.During first 3 days of illnessplatelet count is normal. Thrombocytopenia beginsduring febrile phase and platelet count is progressively reduced during hemorrhagic illness.[11] As per WHO guidelines, thrombocytopenia can be used as a simple diagnostic criteria for DHF.[12] The only accessory laboratory test which supports the diagnosis of dengue is platelet count and it can be roughly estimated by microscopy even in the peripheral laboratories.[13]

Hence the present study is designed to correlate the dengue serological markers with platelet count which, not only helps in identifying and categorizing the patient but also in planning management accordingly, thereby curtailing further progression of disease to its severe forms and thus increasing positive prognosis.

RESEARCH METHODOLOGY

This was a prospective study conducted for a period of one year. The study period extended from January 2019 to 31st December 2019.

STUDY SUBJECT

This study was performed among patients with febrile illness who were clinically symptomatic for dengue infection and referred to the Department of Microbiology, Gajra Raja Medical College,Gwalior for investigation of dengue virus infection during the study period of one year.

INCLUSION CRITERIA

Patients with febrile illness who were clinically symptomatic for dengue infection of all age whose blood samples were received in Department of Microbiology,Gajra Raja Medical College during the study period.

EXCLUSION CRITERIA

- Patients who fail to give consent for the serological diagnosis.
- Patients with autoimmune diseases
- Samples which were exhibiting hemolysis, and whose serum is icteric or showing lipaemia.

STUDY PROCEDURE

All patients with either nonstructuralprotein antigen (NS 1 antigen) positive cases tested by EUROIMMUN Dengue Virus NS1 ELISA kit or dengue immunoglobulin M (IgM) positive cases tested byNIV DENGUE IgM Capture ELISA Kitwere identified and data of febrile patients were collected from different outpatient and inpatient departments of J.A. group of Hospitals. Out of the total 5 ml of collected blood sample, 2ml was kept in EDTA vial and 3ml in plain vial. Sample in EDTA vial was sent for platelet count by fully automated blood cell counter and blood sample in plain vialwere allowed to clot at room temperature (20-25^{o}C) and centrifuged according to the standard serological procedures. The serum separated was stored in refrigerator at 2-8^{o}C and used for serological tests.

RESULTS AND OBSERVATIONS

A total number of 1,762 samples of clinically suspected dengue fever was processed for serological markers of dengue in Department of microbiology during the period of one year from January 2019 to December 2019. Out of the 1,762 total cases, 568 cases were positive for serological tests, Prevalence of dengue seropositivityin clinically suspected dengue patients was 32.22 %In our study out of the 1762 sampletested a total of 568 samples were tested positive for either one or both markers (NS1, IgM) tested. Of the 568 positive serum samples, 249 (43.83%) patients were positive for NS1 only and 211 (37.15%) positive for IgMonly,.Both the markers was detected in the remaining 108 (19.01%) samples.

The present study showed that 77.29 % of dengue seropositive patients were having platelet count below 100000/cc while 22.71% dengue seropositive patients were having platelet count above 1,00,000/cc. In patients having thrombocytopenia 49 patients had platelet count below 20000, 124 patients had platelet count between 21000-51000 and 266 patients had platelet count between 51000-100000.

The present study revealed thatout of the 568 total positive cases of dengue, 439 (77.29%) showed thrombocytopenia. In 249 cases that were positive for NS1 Ag, thrombocytopenia was evident in 197 (79.12%) cases. and when IgM antibodies is found to be positive thrombocytopenia was noted in 151out of 211 (71.56%) cases. When both the markers NS1 Ag + IgMAbwas positive, thrombocytopenia was observed in 91 out of 108 (84.25%) cases.In our study significant association found between serological markers of dengue infection and platelet count. The findings were statistically significant (p value < 0.05) χ^2, DF, p value 7.40, 2, 0.024688.

Table 1: Comparison of platelet count with various serological markers of dengue.

Dengue specific parameter	Total positive serum sample	Platelet count <100000	Plate Count >100000	
NS1 Ag	249	197 (79.12%)	52	χ^2, DF, P Value 7.40, 2, 0.024688
IgMAb	211	151 (71.56%)	60	
NS1 Ag + IgMAb	108	91 (84.25%)	17	
TOTAL	568	439 (77.29%)	129	

On comparison of platelet count with dengue seropositivity, it was revealed that thrombocytopenia [platelet count less than 1 lakh, as per WHO guidelines for DHF] were present in higher number of dengue positive 439 out of 568 [77.29%]cases than dengue negative cases 74 out of 350 [21.14%] cases.These findings were statistically significant (p value < 0.05) (Table. 14) χ^2, DF, p value. 509.69, 1, 0.000003.

Table 2: Platelet count of probable dengue cases

Platelet count	Dengue positive cases	Dengue negative cases	
<1,00,000/ml	439[77.29%]	252[21.14%]	χ^2, DF, p value 509.69, 1, 0.000003
>1,00,000/ml	129[22.71%]	942[78.86%]	
Total	**568**	**1194**	

In our study it was found that out of the 34 cases who had bleeding manifestations, 17 patients had platelet count below 20000/µL, 11 patients had platelet count between 21000 to 50000/µL, 5 had between 511000/µL to 100000 /µL, 01 had platelet count above 100000/µL

CONCLUSION

Dengue and DHF is endemic in more than one hundred countries. South East Asia and Western pacific regions are severely affected bearing 75% of global disease burden. A total of 1762samples were tested and 568 samples are found positive for either one or both the serological markers of dengue virus infection indicating the seropositivity of 32.2% with maximum cases identified during post-monsoon period. The most common clinical presentation was fever followed by headache,nausea and vomiting, abdominal pain and arthralgia myalgia and retro-orbital pain. Rashes and bleeding manifestations also found in some cases. Out568 samples tested positive, 249 (43.83%) patients were positive for NS1 only, 211 (37.15%) positive for IgM only and both the markers were detected in the remaining 108 (19.01%) samples. Among the total positive cases of dengue, 77.29% showed thrombocytopenia. In cases that were positive for NS1 Ag, thrombocytopenia was evident in 79.12% cases. and when IgM antibodies is found to be positive thrombocytopenia was noted in 71.56% cases. When both the markers NS1Ag + IgMAbwas positive, thrombocytopenia was found in 84.25% cases. Potential fatal complications were mostly observed in cases having platelet count below 20000. This study will help clinicians to monitor the progression of dengue fever.

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