Original Research Article

Correlation of serological markers and thrombocytopenia in early diagnosis of dengue infection

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ABSTRACT

Background: Dengue is an acute viral infection with fatal complications. To reduce the disease burden, early and specific diagnosis of dengue hemorrhagic fever or dengue shock syndrome with supportive therapy reduces morbidity and mortality. This study was undertaken to evaluate the efficacy of NS1 antigen and platelet count.
Methods: A total of 200 serum samples were collected from clinically suspected cases of dengue fever. Serum samples were tested for NS1, IgM and IgG and correlated with the platelet count.
Results: Out of 200 samples screened in our study, 100 (50%) were positive for dengue parameters, 100 (50%) were negative. Out of 100 positive cases, 95 (95%) cases were positive for NS1antigen either alone or in combination with antibodies. 84 (84%) cases were exclusively positive for NS1 antigen only. Primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 99 (99%) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 1 case (1%). Out of 100 positive cases, thrombocytopenia was observed in 60 (60%) of patients while 40 (40%) had platelet count within normal range.
Conclusions: Dengue is endemic to Indian subcontinent. Currently no specific antiviral therapy is available. Measuring platelet count and detecting the NS1 antigen plays a vital role in early diagnosis, management and implementing the control measures in community to avoid spread of dengue.

Keywords: Dengue fever, IgG antibody, IgM antibody, NS1 antigen

INTRODUCTION

The present study is designed to correlate the dengue serological markers with platelet count. It not only helps in identifying and categorizing the patients but also in planning management accordingly. Thereby it helps in curtailing further progression of disease to its severe forms and thus it improves the prognosis.

The World Health Organization (WHO) classifies dengue as an important disease affecting public health. The epidemiology and ecology of dengue infection is strongly related with human habits and activities.[1]

Dengue fever is a mosquito-borne viral infection. It causes a severe flu-like illness. It sometimes causes a potentially lethal complication called severe dengue transmitted by bites of Aedes aegypti and Aedes albopictus mosquito.[2]

Dengue fever is caused by any of four closely related serotypes called as DENV 1, DENV 2, DENV 3, and

DENV 4. Symptoms of infection are sudden onset of high fever (103-106°F), severe headache, backache, intense pain in joints and muscles, retro-orbital pain, nausea and vomiting and a generalized erythematous rash. Rash begins 4-7days after the mosquito bite and typically lasts 3-10days.[3] Amongst the 2.5 billion people at risk globally, 1.8 billion or 70% of them live in the Asia-pacific region. Increased urbanization, international trade and travel disseminates both vector and viruses of dengue infection.[4]

The laboratory confirmation of dengue is challenging. Most appropriate tests to confirm the infection depend on the day of illness and whether the infection is primary or secondary. Dengue IgM is usually present between days 5 and 7 of illness. So, a negative IgM before day 7 does not exclude dengue infection. Similarly, a positive test may not indicate a dengue infection. A negative IgM should be repeated during recovery phase. Dengue IgG can be detected in most patients after day 7 of illness. Dengue PCR is useful in early dengue infection, but it is costly and not available everywhere. NS 1 antigen is used in the early phase of dengue infection.[5]

In an outpatient setting in a dengue endemic area, thrombocytopenia in acute febrile illness has a sensitivity of 88% and specificity of 71% in predicting acute dengue infection. It is more useful to exclude than to diagnose dengue infection.[6] Identifying Dengue Specific IgM and IgG is mainstay of diagnosis. Isolation of virus, RT-PCR is time consuming and costly method. Hence identifying IgM, IgG and NS1 specific to virus remains as important diagnostic parameters.[7]

Dengue virus infection produces self-limiting illness to fatal life-threatening complications. Complications include dengue hemorrhagic fever and dengue shock syndrome. Detection of dengue specific NS1 antigen, IgM and IgG antibody detection can be used for early diagnosis. It is essential for effective management of cases to reduce the mortality and morbidity.[8]

During first 3 days of illness platelet count remains normal. Thrombocytopenia begins during febrile phase. Platelet count is progressively reduced during dengue hemorrhagic fever.[9] As per WHO guidelines, thrombocytopenia can be used as diagnostic criteria for Dengue hemorrhagic fever.[10]

Aims and objectives of the study was to identify the dengue specific serological markers, NS1 antigen, anti-dengue IgM and IgG antibodies by ELISA technique and to correlate them with platelet count of probable dengue cases.

METHODS

This retrospective study was carried out for a period of 4 months from September 2017 to December 2017 at Shakuntala Pathology laboratory, Nagpur. A total of 200 blood samples were collected from clinically suspected cases of dengue fever attending various clinicians. Screening for NS1 antigen, anti-dengue IgM and IgG antibodies by ELISA technique was done.

The only non-dengue parameter which helps in predicting complications is platelet count. Hence this study was conducted to identify the dengue specific serological markers and to correlate them with platelet count.

Source of data

200 patients with suspected Dengue Fever at Shakuntala Pathology laboratory, Nagpur.

Study population was divided into two groups

- Under 15-years old patients,
- >15 years old patients.

Inclusion criteria

All the patients with Clinical features suggestive of Dengue infection, later on confirmed by serology were included in this study.

Exclusion criteria

Any other identified specific infection was excluded from the study. Investigations done-

- NS1 antigen
- IgG antibody
- IgM antibody
- Hemoglobin
- Hematocrit
- Total Lecocyte count
- Lymphocyte count
- Platelet count

The results of dengue specific parameters were compared against platelet count.

Statistical analysis

The collected data as well as the proportions and percentages of variables are projected by appropriate tables. As there is no comparative study involved, no significant statistical method was applied.

RESULTS

Table 1 shows, out of 200 samples screened for dengue in our study, 170 (85%) were above 15 years of age while 30 (15%) were of less than 15 years of age.

Out of 200 samples screened for dengue in our study, 112 (56%) were males while 88 (44%) were females as shown in Table 2.

Table 1: Age distribution.

Age distribution	Total no. of suspected cases	%
<15 years	30	15
>15 years	170	85

Table 2: Sex distribution.

Sex distribution	Total no. of suspected cases	%
Males	112	56
Females	88	44

Table 3: Prevalence of dengue parameters.

Dengue parameters	Total positive cases	%
NS1 Ag	84	84
IgM Ab	5	5
IgG Ab	0	0
NS1 Ag + IgM Ab	10	10
NS1 Ag + IgG Ab	0	0
IgM Ab + IgG Ab	0	0
NS1 Ag + IgM Ab + IgG Ab	1	1
Total	100	100

Table 3 shows, out of 200 samples screened in our study, 100 (50%) were positive for dengue parameters, 100 (50%) were negative. Out of 100 positive cases, 95 (95%) cases were positive for NS1antigen either alone or in combination with antibodies. 84 (84%) cases were exclusively positive for NS1 antigen only. In our study, primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 99 (99%) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 1 case (1%).

Table 4: Hematocrit levels.

Hematocrit levels	Total no. of cases	%
Raised	49	49
Within normal range	51	51

In present study, out of 100 positive cases, raised haematocrit values were observed in 49 (49%) of patients while 51 (51%) had hematocrit values within normal range as shown in Table 4.

Table 5: Total leukocyte count.

Total leukocyte count	Total no. of cases	%
<5000/cmm	94	94
>5000/cmm	6	6

In our study, out of 100 positive cases, leukopenia was observed in 94 (94%) of patients while 6 (6%) had total leukocyte count within normal range (Table 5).

Table 6: Platelet count of dengue positive cases.

Platelet count	Total positive cases	%
<1,00,000/ml	60	60
> 1,00,000/ml	40	40
Total	100	100

Out of 100 positive cases, thrombocytopenia of <1,00,000/ml was observed in 60 (60%) of patients while 40 (40%) had platelet count of >1,00,000/ml (Table 6).

Table 7: Dengue parameters and platelet count comparison.

Dengue parameters	Total Positive cases	Platelet count <1,00,000/ml	%
NS1 Ag	84	53	63.09
IgM Ab	5	2	40
IgG Ab	0	0	0
NS1 Ag + IgM Ab	10	5	50
NS1 Ag + IgG Ab	0	0	0
IgM Ab + IgG Ab	0	0	0
NS1 Ag + IgM Ab + IgG Ab	1	0	0
Total	100	60	60

Thrombocytopenia in dengue infections is not an early indicator of severe disease but it helps in predicting the progression of disease. On comparison of platelet count with dengue seropositivity, thrombocytopenia (platelet count less than 1 lakh, as per WHO guidelines for DHF) is seen in more number of dengue positive 60 (60%) cases (Table 7).

DISCUSSION

Out of 200 samples screened for dengue in our study, 170 (85%) were above 15 years of age while 30(15%) were of less than 15 years of age. W K Cheah et al in a one-year retrospective study in Negeri Sembilan in 2010 of 1,466 dengue cases found that the youngest affected was 8 months old and the oldest was 89 years old. The mean age was 32.2 years.[5] Out of 200 samples screened for dengue in our study, 112 (56%) were males while 88 (4415%) were females.

Kulkarni SK found that there were 95 (63.33%) males and 55 (36.66%) females, with a male to female ratio of 1.73:1.[11] Mohan Kashinkunti found that 54% were males and 46% were females.[12]

In our study, out of 100 positive cases for dengue, 95 (95%) cases were positive for NS1antigen either alone or in combination with antibodies. 84 (84%) cases were exclusively positive for NS1 antigen only. During first few days of illness, NS1 Ag circulates at high level in blood. Hence positive NS1 antigen test in a patient indicates acute phase of illness.

Dengue specific IgM can be detected in blood only after 3 to 5 days of illness; hence it cannot be used as an early diagnostic marker.

SN Kanthikar et al found that out of suspected 358 serum samples tested at central clinical laboratory, 135 cases were positive for NS1, IgM or IgG. Majority of 57 (63.33%) cases were positive for IgM, followed by NS1 and IgG with 45 (33.33%) and 7 (5.1%) cases respectively.[13]

In our study, primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 99 (99%) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 1 case (1%). Saroj Golia et al reported 57.4% primary dengue infections and 42.6% secondary dengue infections in their study.[14] In present study, out of 100 positive cases, raised haematocrit values were observed in 49 (49%) of patients while 51 (51%) had hematocrit values within normal range.

For DHF, MS Ahmed Khan et al observed a greater variation of Hb in both groups. It was most evident from 4th to 6th days with the highest values in patients aged 15 years old or more. For SD, there is a slight variation between the groups.[15] In our study, out of 100 positive cases, leukopenia was observed in 94 (94%) of patients while 6 (6%) had total leukocyte count within normal range.

Leukocytosis was observed by Ahmed Khan et al in patients with the CD in the first days of the disease, followed by leucopenia. Leucopenia was more pronounced in the under 15-year-old age group.[15] In our study, out of 100 positive cases, thrombocytopenia of less than 1 lakh was observed in 60 (60%) of patients while 40 (40%) had platelet count of more than 1 lakh.

Thrombocytopenia in dengue infections is not an early indicator of severe disease but it helps in predicting the progression of disease. On comparison of platelet count with dengue seropositivity, thrombocytopenia (platelet count less than 1 lakh, as per WHO guidelines for DHF) is seen in more number of dengue positive 60 (60%) cases.

In CD, thrombocytopenia was observed by Ahmed Khan et al in both age groups from the 4th day of the disease. The DHF and SD forms started with thrombocytopenia and pronounced variations were recorded throughout the evolution in both age groups.[14]

Kotresh Doddamane et al found that platelet count at the time of admission was below 10000 in 7.5% of the patients and above 100000 in 22.5% of the patients.[16] Kotresh Doddamane et al found that v45% of the patients were positive for IgM anti dengue antibodies and 55% were positive for both IgM and IgG antibodies.[16]

CONCLUSION

Nonspecific symptoms of dengue infection during its early phase necessitate the need for its differentiation from other febrile illness. Platelet count of dengue positive cases were significantly lower than the dengue negative cases. This helps the clinician for early prediction and monitoring of the dengue positive case for the development of DHF and DSS thereby reducing the complications. The impact and cost-effectiveness of NS1 Ag in the evaluation of acute fever and the optimum outpatient management of dengue infection and admission criteria should be given priority by the primary care and emergency departments.

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